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THE USE OF PITUITARY IMPLANTS AND PITUITARY EXTRACTS FOR OBTAINING AMPHIBIAN EGGS OUT OF SEASON

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Instructor Embryology, Columbia University

A difficulty which confronts many embryologists and experimental zoologists is that of obtaining material during the autumn and winter months. To be sure, one can always use the chick and eggs of certain fishes, but the experiments which can be carried out on these forms are limited. The amphibian egg which is ideal in many respects for embryological work is usually available only during a relatively short period of the year. By the use of the pituitary gland and extracts it is possible, however, to induce frogs, newts and salamanders to lay their eggs from October until June. The technique is relatively simple and has been used by students in our laboratory with amazing success.

The effects of the pituitary have been examined by Wolf, 1929, who found that by implanting the pituitary body of the frog into the (*Continued on page 7*)

M. B. L. Calendar

SATURDAY, JULY 1, 8:30 P. M.

M. B. L. Mixer.

WEDNESDAY, JULY 5, 8:00 P.M.

Seminar: Dr. G. W. Prescott: "Some Effects of blue-green Algae on Lake Fish."

Dr. Hugh P. Bell: "Distribution and Ecology of the Marine Algae of the Maritime Provinces of Canada."

Dr. W. R. Taylor: "Distribution of Newfoundland Algae."

FRIDAY, July 7, 8:00 P. M.

Lecture: Dr. G. H. Parker: Neuro-Humoralism and Nerve Transmission.

RESEARCH FACILITIES AT THE SCRIPPS INSTITUTION OF OCEANOGRAPHY

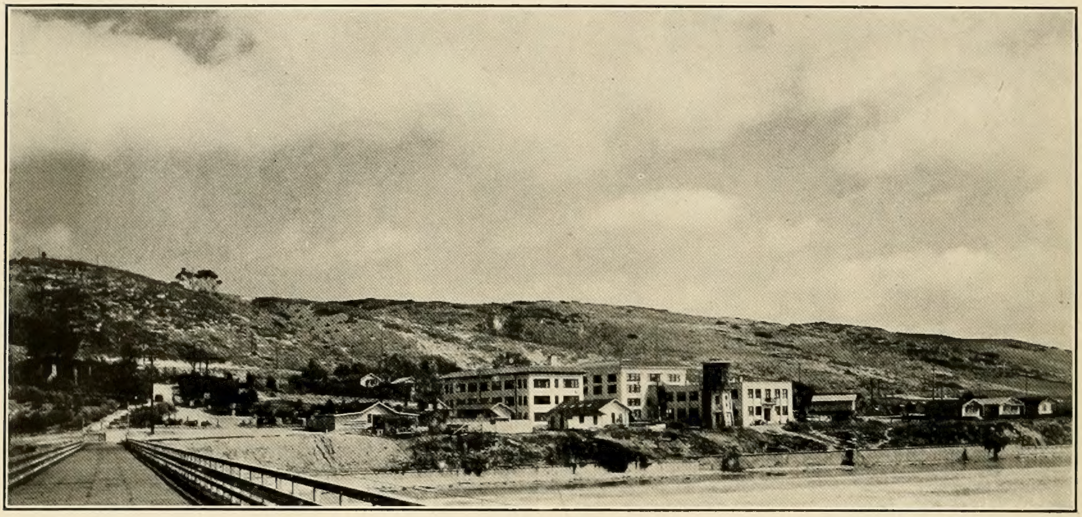
DR. THOMAS WAYLAND VAUGHAN

Director of the Institution

The development of the Scripps Institution of Oceanography has been slow, having extended over fully forty years. The events which led to the establishment of the Scripps Institution for Biological Research and the early history of that Institution have been excellently presented in a bulletin entitled, "The Marine Biological Station of San Diego, its history, present conditions, achievements, and aims," by Dr. W. E. Ritter[1].

Subsequent to the publication of the article by Dr. Ritter and prior to my succeeding him as director of the Institution there were two notable improvements in the Institution. One was the erection of a library-museum building, 60 ft. x 60 ft., which contains two floors and about a three-quarter basement. Two tiers of book-stacks can be installed on each of the two main floors of the building, giving a library capacity of about 50,000 volumes.

TABLE OF CONTENTS



THE SCRIPPS INSTITUTION OF OCEANOGRAPHY

View of the three principal buildings looking northwest. In the center is the Library-Museum building; at the right is part of the new laboratory, Ritter Hall; at the left behind the Museum-Library building is the old George H. Scripps Laboratory. At the extreme left may be seen part of the pier.

The other improvement was the construction of a re-enforced concrete pier, 1,000 ft. long and 20 ft. wide. Therefore, at the time when I became director of the Institution on the first of February, 1924, the land of the Institution consisted of a 177-acre tract on the sea front about two miles north of the village of La Jolla. There were, on this land, one laboratory building, two floors, 75 x 48 ft.; the library-museum building and the re-enforced concrete pier, which have been mentioned; a wooden aquarium, 24 x 48 feet, with 18 tanks; several service buildings and garages, temporary structures, and 24 wooden cottage residences. In September, 1925, the Institution purchased a boat of the purse-seiner type, 64 ft. long, 18 foot beam, about 50 tons gross, and it was equipped to work to a depth of about 1,000 fathoms. The boat is supposed to have a cruising radius of about 1,000 miles, but no attempt has been made to use it for cruises of more than about 200 miles radius.

Nearly all of the expense of the acquisition of the land and the construction of the buildings on it was borne by Miss Ellen Browning Scripps. Mr. E. W. Scripps, however, helped defray the expenses of some of the construction, and he donated the boat to the Institution.

The expenses of operation were divided between the Scripps family and the State of California. Miss Scripps contributed $9,000, and Mr. E. W. Scripps, $5,000, per year. Revenue of about $7,500 per year was derived from the Institution's houses and its supply department. The University of California contributed $22,500 per year.

The general set-up of the Institution when I became its director was as has been indicated. President Campbell proposed to Miss Scripps that, beginning July 1, 1925, she and the University of California jointly make an annual step-up of $5,000 a year in the income of the Institution, each to contribute $2,500. This policy was continued until there had been an increase of $35,000 per year in the Institution's income. Besides the income above indicated the Institution has received a considerable number of special contributions of significant size. The principal of these was a fund for the meteorological investigations, raised cooperatively by the organizations interested in hydro-electric power development in the State. These contributions were from the Department of Water and Power, City of Los Angeles, and a number of commercial corporations.

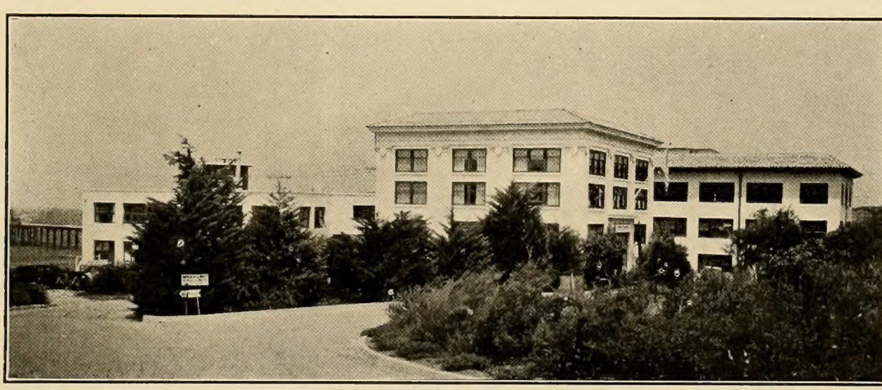
In 1929 it was decided to add a new laboratory building to the Institution's equipment, and for this purpose $120,000 were raised by $40,000 contributions from each of the following: the State of California through the University, Miss Scripps, and the Rockefeller Foundation. Work on the building was begun in March, 1931, and during September of the same year it was ready for use. This building is a three-story fire-proof re-enforced concrete structure. It is 100 ft. long and 46 ft. wide. For the biennium which began July

1, 1931, the State of California made to the University a special appropriation of $40,000 for the renovation and improvement of the old buildings and grounds of the Institution.

The statements above made indicate the general nature of the physical set-up of the Institution at present, how the set-up was brought about, and what the present resources of the Institution are. But nothing has been said regarding the scientific purpose which was behind the development.

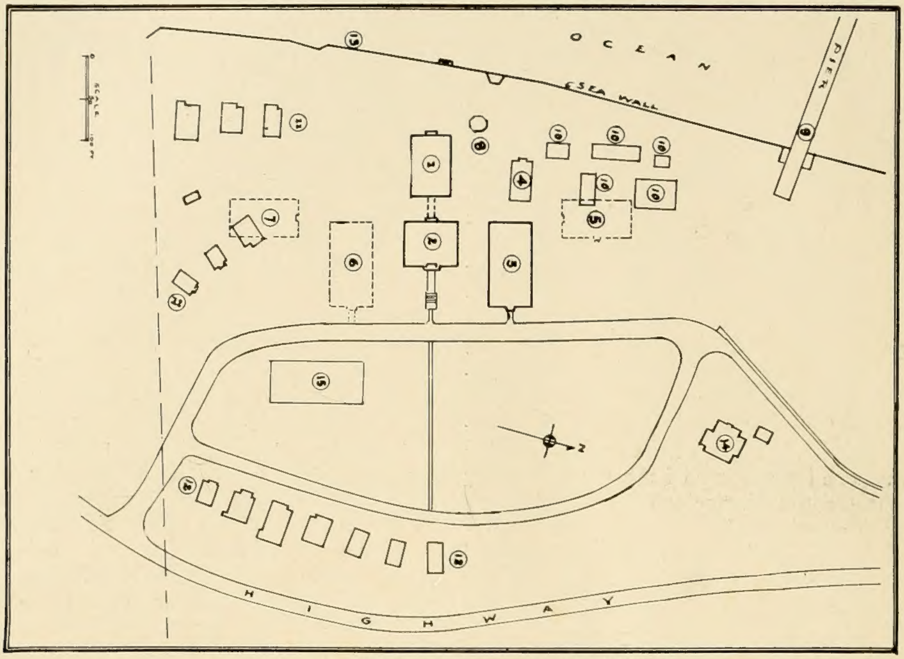
The Institution at first was primarily one for biological research, but very soon after its establishment important investigations in dynamical oceanography, particularly those of Dr. George F. McEwen, were undertaken, because it was recognized that knowledge of the physical features of the environment was necessary in order to understand the conditions of life of the various marine organisms. The lower floor of the old laboratory building, the George H. Scripps Laboratory, was supplied with running salt water, gas, and electricity, and it was adapted for general morphological work and experimental work which did not require controlled conditions. As is known by everyone who is familiar with the history of the Institution, numerous important scientific researches were conducted at the Institution during the incumbency of Dr. Ritter.

Before Dr. Ritter's retirement on June 30, 1923, the administrative officers and regents of the University of California and the interested members of the Scripps family decided to convert the Institution from one for biological research into one for oceanographic research. Therefore, when I came to the Institution on February 1, 1924, a change in the Institution's policy was initiated. An endeavor was made to develop a broad program of oceanographic research which would be in line with the generally recognized scope and functions of an oceanographic institution. In the lines of research which had been pursued under the incumbency of Dr. Ritter, it was decided to make only one significant change. Although it was recognized that the investigations of geographic races and heredity in the deer mice, *Peromyscus*, by Dr. F. B. Sumner were of great importance, really an honor to the Institution with which Dr. Sumner was connected, the research was scarcely germane to an oceanographic institution. Dr. Sumner himself suggested that, after his work on *Peromyscus* had been brought to a logical stopping place, he should transfer his activities to problems in fish biology, which had been subjects of investigation by him before he undertook the *Peromyscus* project. Dr. Sumner's suggestion was adopted by the appropriate University officials. The investigations in dynamical oceanography under Professor McEwen, the studies of phytoplankton by Professor W. E. Allen, and the work on the zooplankton, especially copepods, by Professor Esterly, were continued. As soon as it was practicable to do so, the work in marine meteorology was expanded, and investigations of the chemistry of sea water were established as one of the definite projects of the Institution, with Dr. E. G. Moberg in charge. The study of marine bottom deposits and the complex of associated physical and chemical problems in the ocean, in charge of T. W. Vaughan, was undertaken as soon as circumstances permitted. Since micro-organisms, bacteria and the lower forms of plant life, perform important rôles in the metabolism of the sea, investigation of those organisms was initiated, first in charge of Dr. A.



VIEW OF THE THREE PRINCIPAL BUILDINGS LOOKING SOUTHEAST FROM THE PIER

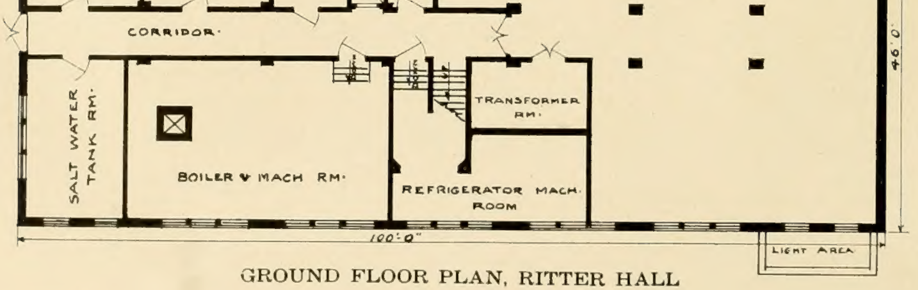
At the left is the new building, Ritter Hall; in the center is the Museum-Library building; at the right behind the Museum-Library building is the George H. Scripps Laboratory.



PLAN OF BUILDINGS AND GROUNDS

H. Gee, later in charge of D. C. E. ZoBell. Investigations of the physiology of marine organisms with reference to their oceanic environment, in charge of Dr. D. L. Fox, were added. It is generally recognized that the adaptations of organisms to their environment are fundamentally physiological. Therefore, in order to get at the more fundamental principles of such adaptation it is necessary to know the organism structurally, the environment in which it lives, and the organic processes which are necessary for the organism to continue its existence.

The lines of research being prosecuted at the Institution may, therefore, be categorically stated as follows: dynamical oceanography and marine meteorology, including solar radiation and the penetration of radiant energy into sea-water; the chemistry of sea-water; biology (bacteriology; phytoplankton; foraminifera, their life history and relation to marine sediments; biology of fishes, largely physiological; physiology of marine organisms, with special reference to adaptation to

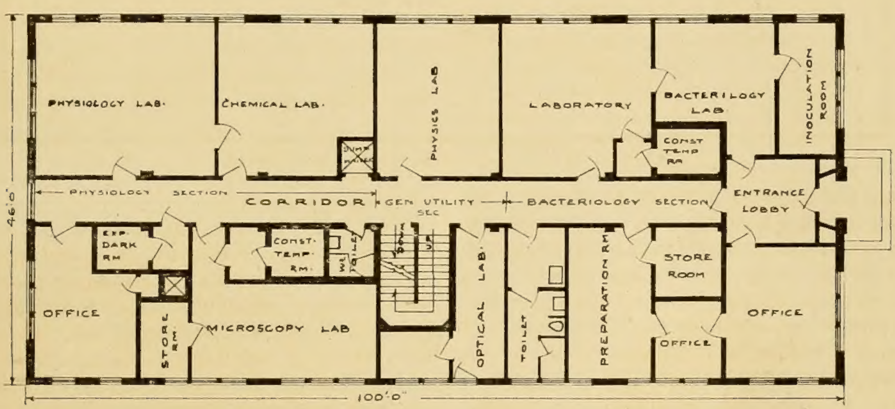


GROUND FLOOR PLAN, RITTER HALL

the marine environment); geological processes, especially marine bottom deposits. The Institution has on its grounds one of the seismological stations established in connection with the study of seismology in southern California under the auspices of the Carnegie Institution and the California Institute of Technology. Each line of oceanographic work interlocks with other lines. Therefore, although it has been necessary more or less to sectionize the work of the Institution, because of the need for skill in the use of special disciplines, the work of the Institution is really set up as a series of interlocking projects.

In addition to what is done by the resident members of the staff of the Institution and by several non-resident assistants, the Institution derives great benefit from cooperative relations with a number of other institutions, especially various governmental organizations. The assistance received from the United States Navy, the Coast and Geodetic Survey, and the Bureau of Lighthouses, has been invaluable. It includes the collection of data and samples of water and bottom deposits from a large part of the northeastern Pacific. The Institution has also received aid from the Grace and Los Angeles Steamship Lines and from many other sources.

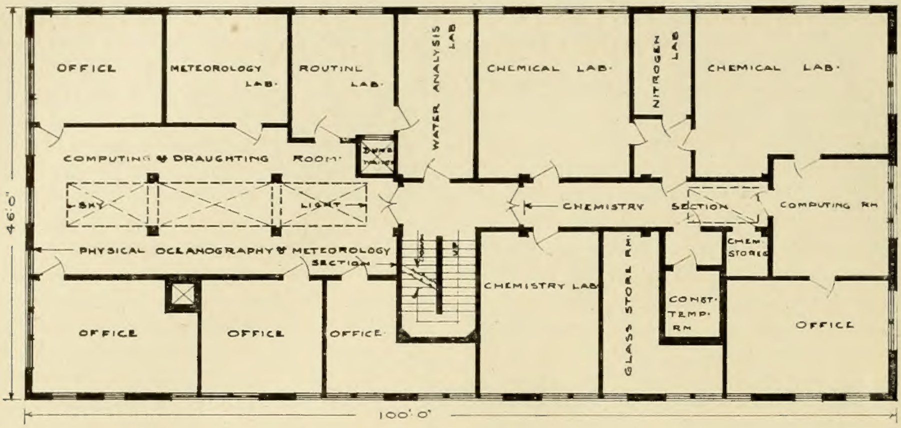
The account of the purpose and organization of the Institution has been rather fully stated because they have influenced the recent construction and other improvements of the Institution's facilities for scientific research. The new laboratory building, which has been appropriately named Ritter Hall, has provisions in it for several different kinds of research. On the top floor, east end, there are three research chemical laboratories, a nitrogen room, an alcove for colorimetric work, and a small laboratory for routine determinations, such as salinity and hydrogen ion concentration. There are also one rather large office, a drafting and computing room, two store rooms, and one room in which the temperature may be kept constant as desired, from 0° to 40° Centigrade. At the west end of the top floor there are rooms for dynamical oceanography and marine meteorology, including a large computing room extending the whole length of that section of the building. In connection with the work in marine meteorology there are in the rooms on the top floor the recording devices of instruments, such as for solar radiation, which are exposed on the roof of the building. On the roof there is also a pent-house, in which the water distilling and ventilating fans are installed. On the second floor, east end, there are for the bacteriological investigations five rooms, in addition to a constant temperature room. Just west of the bacteriological suite there is a long room intended for spectrometric purposes, and a large laboratory which was intended as a special physics room. At present this room is being used as a biological laboratory, but such use is not intended to be permanent. At the west end of the second floor is the physiological suite. It comprises, besides the office, one large experimental laboratory supplied with salt water, a large chemical laboratory, and a large microscope room. There is also in this suite a dark room and a large constant temperature room.



FIRST FLOOR PLAN

On the ground floor, there are two rooms intended for the large-scale cultures of marine organisms; a photographic suite composed of four rooms; the machinery room and transformer vault; and a room for the storage of oceanographic equipment. On this floor, there remains considerable unassigned space, a part of which at present is in use as a receiving room and another part, as a carpenter shop.

By means of the special State appropriation of



SECOND FLOOR PLAN

$40,000, the George H. Scripps Laboratory, 75 x 48 ft., has been remodelled. A little less than half of the lower floor is devoted to fish biology, and about three and a half unit rooms are devoted to marine sediments. There are on the lower floor three unassigned rooms which may be used by visiting investigators or by staff members. These rooms are supplied with running salt and fresh water, compressed air, gas, and electricity. On the upper floor, there are the Director's offices, the seminar room, and an unassigned room which may be used either as an office or as a laboratory for work which does not require experimentation. On this floor there are also the laboratories for phytoplankton, the offices of fish biology, and two laboratories for those studies on foraminifera which do not require experiments on living organisms.

As a part of the renovation and improvement made possible by the special State appropriation of $40,000, the old salt water system of the Institution was replaced by a new system which consists of a 60,000 gallon re-enforced concrete tank, the concrete and covering cement being designed to prevent rusting of the re-enforcing. The water for the tank is brought from the west end of the pier, nearly 1,000 ft. from the beach line, through pure lead pipes, and from the tank water is distributed through pure lead pipes to the physiological section of Ritter Hall, the aquarium, and the lower floor of George H. Scripps Laboratory. The system appears to be excellent.

Ritter Hall is intended for investigations in dynamical oceanography, the physical properties of sea water, the chemistry of sea water, biochemistry, bacteriology, and general physiology. The laboratory is primarily one for physics and chemistry for those kinds of biological investigation which require either biochemical or biophysical methods. The equipment of George H. Scripps Laboratory is, for the kind of work which has been indicated, at present adequate.

Since library facilities are essential for research, it will be said that the Institution's library now contains 13,000 bound volumes, about 23,000 reprints, and many atlases and charts. An improvement expected in the near future is the replacement of the gasoline engine at present on the Institution's boat *Scripps* by a Diesel engine. The funds needed for this change have been subscribed.

The space available at the Scripps Institution purposely exceeds that which is needed by the Institution's permanent staff which works throughout the year. The Institution can receive visiting scientists for virtually any kind of oceanographic research. There are special facilities for a number of different kinds of biological work. The number of visitors who can be accommodated is approximately 25, that is, about 25 in addition to the residential members of the staff. The new equipment at the Institution first became partially available for use during the summer of 1932. The reconstruction and improvement program had not been completed at the beginning of the summer. Therefore, it is only at the end of the summer season of 1932 that full utilization of the facilities of the Institution has become possible. The Institute desires to be of service to the various branches of oceanographic research and, insofar as it has facilities, it will welcome those who are working on the biological, physical, chemical, and geological aspects of oceanography.

[1] Univ. Calif. Publ. in Zool., vol. 9. pp. 137-248, pls. 18-24, two maps, March 9, 1912.

THE USE OF PITUITARY IMPLANTS AND PITUITARY EXTRACTS FOR OBTAINING AMPHIBIAN EGGS OUT OF SEASON

(Continued from Page 1)

lymph sac of the female ovulation could be stimulated. Noble, 1931, refers to the use of the pituitary for egg-laying in other amphibia. See also Buyse and Burns, 1931 ; Adams, 1930.

RANA PIPIENS

Starting from Wolf's experiments the following technique has been worked out in our laboratory. A male and female frog in healthy condition are kept in an aquarium jar with a few inches of spring water and each is given two implants of frog pituitary daily for three or four days. Almost all frogs so treated go into amplexus, and normal ovulation and fertilization take place. The frogs used as recipients of the implants should be medium-sized frogs which have not been kept in the laboratory too long. When they have been kept two or three weeks at room temperature they do not respond so readily. Our frogs come from Vermont and can be obtained throughout the winter.

The pituitary can easily be obtained by cutting off the head of a frog and cutting through the floor of the brain case on either side. The pituitary will usually adhere to the floor of the brain case and can be picked up with a pair of forceps and inserted through an incision in the skin of the host. We have found that by making a cut through the skin just back of the foreleg the pituitary can be pushed posteriorly into the lymph sac underneath the skin and will not slip out when the frog is returned to the aquarium. This incision remains open and all implants may be introduced through it. It is important to use two pituitaries for each operation if the eggs are to be fertilized. If only one is used, the eggs may be deposited without being fertilized by the male, and there is greater variability in the results. There is also a seasonal variation, and ovulation is more easily induced in the spring.

Recently Mr. R. Rugh has simplified this procedure by injecting into the abdomen of the female an aqueous extract of the pituitary. The following account is from his unpublished experiments. The extract is prepared by grinding the pituitary with a glass rod in the bottom of a tube. Two pituitaries are used in about 2 cc. of distilled water. A single injection of this extract is usually sufficient to move the eggs into the uterus. After the injection the frogs are kept at room temperature and after a period of from 24 to 48 hours the eggs are stripped into a large dish containing a thin layer of sperm suspension. In the fall and early winter the eggs appear after about 48 hours. In the spring from 3 to 24 hours after injection.

The sperm suspension is prepared by macerating the testes in spring water. The males do not have to be injected for active sperm. This sperm suspension is allowed to stand for about one half-hour before using. Two points should be emphasized. First, that the sperm suspension should be allowed to stand for some time before using; and second, that the sperm suspension should form a thin layer and the eggs be stripped from the female directly into the suspension. If fresh sperm or large volumes of water be used, the jelly of the eggs swells before fertilization occurs. About 20 minutes after insemination the dish may be filled with spring water. The jelly then swells and the fertilized eggs rotate within their membranes.

After the female has been injected and the eggs accumulate in the uterus, the frog may be kept in a cold room at 9°-11° C. and the eggs may be used as needed. At room temperature the eggs are usually laid and then cannot be fertilized. However, the eggs remain fertile in the uterus for several days if the female is kept at a low temperature.

The aqueous extract of the pituitary is stable and can be kept on ice for months. Pituitaries from females are more effective than those from males because they are larger.

Many attempts have been made to substitute sheep's pituitary and Antuitrin-S for the frogs' pituitary, but without success. On the other hand both sheep's pituitary and Antuitrin-S are effective in newts and salamanders.

NEWTS

In a similar manner ovulation may be stimulated in an American newt, *Triturus viridescens*, and in a Japanese newt, *Triturus* (Molge) *pyrrhogaster*. The female usually carries sperm, and if the newts be treated in the fall and winter, fertilized eggs are laid without implanting males. We have obtained fertilized eggs from the Japanese newt as late as May. Some females lay unfertilized eggs, which may be inseminated artificially with sperm from the male. The sperm are removed from the vasa deferentia and placed in a few drops of Ringer's solution and the eggs are dipped into the concentrated sperm suspension. The eggs can be removed from the female by pressure on the abdomen or can be dissected out of the uterus. In either case they must be

immediately dipped into the sperm suspension and then placed on the bottom of a moist dish. Later water may be added and the membranes then swell.

Frogs' pituitary, sheeps' pituitary or Antuitrin-S may be used to obtain eggs from the newt. Single or double implants of frogs' pituitary underneath the skin of the female for a few days are sufficient to cause ovulation. The animals are kept in a small aquarium with Elodea and the eggs are deposited on the leaves of this plant.

Daily injections of extract of sheep's pituitary as prepared by Parke, Davis and Company will cause the Japanese newt to lay. About 0.3-0.5 cc. is injected beneath the skin daily or into the abdomen for three or four days. The newts, if well fed and healthy, invariably lay. The eggs will be fertilized or unfertilized, depending upon whether or not the female carries the functional sperm. Ogilvie, 1933, has used Antuitrin-S with the same success.

Triturus pyrrhogaster is a very hardy newt which lives well in the laboratory and can be obtained in large quantities from New York aquarium supply houses. It is much larger than *Triturus viridescens* and lays many more eggs. The eggs are very valuable for cleavage studies and, in later stages, for transplants.

SALAMANDERS

The Mexican axolotl, *Amblystoma mexicanum*, will also lay eggs after injection of sheep's pituitary or Antuitrin-S. If the females be injected with about 1 cc. of these extracts daily, they will deposit their eggs, but the eggs will not be fertilized. If fertilized eggs are desired, males only must be injected with about 1 cc. daily for three or four days. They will then deposit spermatophores and the females will pick them up without injection, and lay fertilized eggs. The animals must be well fed. We have been using chopped beef liver a few times a week. The same females may lay more than once a year, by keeping several pairs eggs can be obtained from October until June.

The eggs are particularly useful for experiments where it becomes necessary to remove the egg membranes and jelly before gastrulation. The jelly may be completely removed with two pairs of sharp forceps, leaving only the thin vitelline membrane which need not be removed for experiments with vital stains. It is much more difficult to work with the frog's or newt's egg before gastrulation because of the large amount of jelly in the former and the tough membranes of the latter.

All of the forms above-mentioned are easily obtainable and inexpensive except the Mexican axolotl. However, the latter lives well in the laboratory, and when once a stock has been obtained, they supply large numbers of eggs. The sheep pituitary extract was supplied by the research department of Parke, Davis and Company. Antuitrin-S can be obtained from the same company or from local drug stores.

Wolf, O. M.—1929—Proc. Soc. Exp. Biol. Med.XXVI, 692-693

Noble, G. K.—1931—The Biology of the Amphibia. New York, 299-300

Buyse, A. and Burns, R. K., Jr.—1931—Proc. Soc. Exp. Biol. Med.XXIX, 80

Adams, A. E.—1930—Anat. Rec.XLV, 250

Ogilvie, A. E.—1933—Proc. Soc. Exp. Biol. Med.XXX, 752

SPECIAL APPARATUS AND TECHNICAL SERVICES

The more expensive and delicate apparatus owned by the Marine Biological Laboratory has been segregated and is separately administered. It is especially cared for in the hope that individual problems of our investigators may be approached with the least delay and as directly as possible. Information concerning these devices and technical supplies for research may be secured through the Apparatus Office, Room 216, in the Brick Building. The supervision of such special apparatus and technical supplies remains in charge of Dr. Samuel E. Pond, and his staff of assistants.

It is important to point out to investigators, particularly those arriving at the Marine Biological Laboratory for the first time, that it is necessary to make arrangements for scientific apparatus and technical supplies in advance of their need. The Laboratory is only able to maintain an adequate technical service by being kept in touch with individual needs. To this end an "Application for Research Accommodations" has been sent to each investigator in which provisions was made for special preferences or requisitions for supplies of a technical nature. From information supplied by these applications preliminary preparations for the season have been made. Such requests for special apparatus and general equipment have also been used as a guide to the location of the more permanent and semi-permanent laboratory devices. For certain special problems scientific apparatus has been re-

served on one of several plans which have been devised for the convenience of investigators. Those individuals who have not taken advantage of the preliminary notice or who have not inquired about special provisions may have been handicapped upon their arrival and have thereby been delayed in starting their work.

The Laboratory is in possession of a fairly complete equipment of physical instruments but with increasing demands and more technical requirements it is difficult to make the best distribution of apparatus to fit peculiar and individual requirements. Since it is desirable to make the special apparatus generally available and to avoid restriction to a small number of persons, reservations for certain devices are arranged to suit particular periods of use. Where it is impractical for investigators to bring with them certain special apparatus the necessary equipment may be reserved for a required period but in such cases a fee will be charged for such use. Arrangements for EXCLUSIVE LOANS for a season or less must be arranged through the Apparatus Office, Room 216, in the Brick Building.

Certain apparatus more generally available may also be rented where more or less personal use is required. Microscopes are reserved for the period of residence, the fee depending upon equipment and device. Special microscopes and many accessories are not usually available, including binocular, monobjective microscopes of the highly specialized or research type; aplanatic condensers; camera lucidas, demonstration oculars, etc. These devices are customarily transferred by investigators from their own institutions with personal supplies.

Students in courses are supplied by the staff in charge of their instruction. Special apparatus cannot be loaned otherwise. Beginning investigators may arrange for supplies through the investigator with whom they are working but must in such cases refer such special requests to the Apparatus Office as require technical assistance. As far as possible, use of special apparatus is fitted to individual requirements, but in most cases allowance for use with others must be made.

The use of certain apparatus, such as analytical balances etc., continually requires the attention of investigators. Both individual assistance in the maintenance of such instruments and personal responsibility for care, cleanliness, and stability are essential to continue these devices in operating condition. Instruments of precision are located at advantageous points but weights and special supplies are not issued for general use. It has been found necessary to provide weights, or special supplies for such work only to individuals who it is expected will arrange for their return in good condition as soon as work permits. To those who use balances, scales and balance rooms particularly, a word of caution may not be amiss concerning the care necessary to avoid damage to delicate parts. An even greater care and more than usual cleanliness of such equipment is essential not only to one's own success but to that of others.

TECHNICAL SERVICES

Provision for part of this season has been made for certain technical services. Arrangements for these will be made through the Apparatus Office.

Technical operation of X-ray equipment has been arranged after July 5th, continuing through August. Special problems involving X-radiation, radium, etc., will necessitate advance arrangements and some rotation or schedule in order to avoid loss of research time.

Glassblowing, as in preceding summers may be arranged during the residence of Mr. James D. Graham, of the University of Pennsylvania, through July and August. Necessary repairs to certain kinds of glass can be done fairly promptly and a limited amount of new work may be arranged. Engraving of glassware requires a greater allowance of time since the work is partly done out of town. Technical work of this kind is done at cost of materials and labor.

Photography may be done by individuals through loans of special apparatus. During part of the present season technical assistance may be arranged and supplies secured if sufficient advance notice is received by the Apparatus Office. Lantern slides, the development of research films, plates and prints may be arranged at the cost of materials and labor, but amateur work cannot be undertaken. Dark room space is limited chiefly to research problems although provision for photographic dark rooms for limited periods may be arranged from time to time. Chemicals and photograph supplies cannot be supplied except to investigators who have made application for them. since local stocks are usually inadequate to meet other than technical needs.

To those who contemplate returning subsequently to the Marine Biological Laboratory it will be a material help to consider working conditions more or less peculiar to this Laboratory and to arrange with Dr. Pond any special allotments of apparatus or supplies for future periods of residence for scientific work.

A SIMPLE APPARATUS FOR THE MEASUREMENT OF RADIATION INTENSITIES

Roberts Rugh
Instructor in Zoology, Hunter College

Non-selective radiation measurements are usually made with a blackened thermopile and galvanometer. With radiation measurements covering a range of 10° C. as measured by ordinary thermometers, the thermopile method is open to considerable criticism.

A simple instrument has been described by Hall (Ecology-13:214) in which two parallel thermometers are exposed to light, one thermometer being blackened with black Duco enamel and the other whitened with zinc oxide. Hall reports that this apparatus compares favorably with the Macbeth Illuminometer, having a correlation coefficient of +0.9805 ±0.0039. Using a Fahrenheit thermometer he reports that 1°F. equals 1,000 foot-candles. The disadvantage pointed out was in the measurement of low light intensities.

With the aid of Mr. Lester Boss of the Marine Biological Laboratory at Woods Hole, an apparatus has been devised which eliminates some of the criticisms of Hall's apparatus. A metal box measuring 5½ x 3½ x 2¼ inches was constructed to act as a water chamber containing within itself a smaller closed box of the same material. Two high precision thermometers which can be read to 0.1°C. accurately are placed with their mercury bulbs in the smaller air-tight box. The thermometers lie parallel to each other and about 1¾ inches apart. The thermometer blackened with optical black to a point above the mercury bulb is shielded from the untreated thermometer by a reflecting metal surface and is exposed to the outside through a window of Heat Resisting Clear Corex D (Corning) Glass. Quartz would be an improvement but the gain would hardly justify the additional cost. Beckmann thermometers could be used to advantage if the temperature range considered is well within the limits of the Beckmann scale. The inner, air-tight metal box measures 3¾ x 2¾ x 1½ inches and is soldered into place. The inlet and outlet tubes for water circulation are located at one end of the box and at opposite corners.

In using this apparatus it is necessary to allow the shielded thermometers to come to equilibrium in the running bath for a considerable period in order to determine the particular characteristics of the thermometers. If the bath temperature is quite different from the original temperature reading of the thermometers, more time should be allowed to attain equilibrium. All radiation measurements should take into account these thermometer characteristics. Radiation intensities within the range of 0.0-5.0°C. can be made within 10-15 minutes, but intensities comparable to sunlight require half an hour to attain equilibrium. The initial response of the exposed thermometer which is perceptible is a matter of 2-3 seconds at moderate intensities.

The temperature difference of the two thermometers with the apparatus exposed to direct sunlight at noon one day last August was 32.20° C., while exposure to the direct sun at a corresponding time of day during January gave 19.60° C. difference. The apparatus will respond to roomlight radiations on sunny days particularly with Beckmann thermometers, which can be read closer than 0.1° C. Numerous readings indicated the roomlight to be equal to about 0.1% of the direct sunlight.

The radiation transmission characteristics of some Corning Glass filters of different spectral values taken at Woods Hole last summer and the same filters re-calibrated with the same apparatus but a different pair of thermometers during January, is given below to indicate how accurately the apparatus can reproduce intensity measurements. In each case the apparatus was clamped into position beneath a G.E. Type S1 Sunlamp, and the filters were interposed at a constant distance of six inches above the apparatus. The radiation intensity of the entire arc of the Sunlamp varies tremendously with a shift of a few inches in any direction, so it was necessary to have both lamp and thermometers clamped into a permanent relationship to each other. The transmission characteristic of each glass filter was calculated in respect to the total transmission of the Sunlamp as 100%. It must be admitted that the spectral energies of the Sunlamp change with age, but this factor is negligible within the time limits of the observations made. The distance from the Sunlamp varied by 12 inches in August and January, hence the "100.0%" value of the Sunlamp was 15.00° C. in one case and 4.49° C. in the other. Nevertheless, the transmission characteristics of the various filters were surprisingly close as expressed in percent. of total energy available.

Light Condition	August Transmission %	January Transmission %	Difference
G. E. Type S1 Sunlamp	100%	100%	00.0%
Filter No. 774	91.59%	91.65%	+0.06%
Filter No. 970	92.59%	92.13%	—0.46%
Filter No. 980	85.99%	87.41%	+1.42%
Filter No. 385	80.20%	78.54%	—1.66%
Filter No. 349	61.92%	60.98%	—0.94%
Filter No. 254	59.60%	59.19%	—0.41%
Filter No. 428	34.76%	33.40%	—1.36%
Filter No. 401	20.13%	20.04%	—0.09%
Filter No. 597	11.51%	10.96%	—0.55%
Filter No. 396	10.73%	9.86%	—0.87%

To test the advantage of blackening the radiation absorbing thermometer, some observations were made with the blackened thermometer shielded and the untreated thermometer exposed to the radiations. With radiations measuring 19.6°C. under normal conditions, with the thermometers reversed, the temperature differences of the two thermometers was only 6.5°C. This indicated that blackening of the bulb increased the efficiency of the absorbing thermometer by approximately 300%.

The obvious disadvantage of this apparatus is its slow response as compared with the electrical methods. However, it must be remembered that with such great intensities and with isolated spectral radiations, this non-selective method overcomes some of the criticisms aimed at thermopiles and photo-electric cells. Filter No. 254 is an infra-red transmission filter; No. 401 is monochromatic green; No. 597 eliminates all but the visible purple and some ultra-violet and a little infra-red; while No. 970 transmits everything from about 2900 A° units deep into the infra-red. The total cost of the apparatus is negligible and the accuracy with which results can be reproduced suggest its practical use.

A SIMPLE DEVICE FOR THE STUDY OF SMALL OPAQUE OBJECTS

DR. ANNA R. WHITING

Professor and Head of the Department of Biology, Pennsylvania College for Women

The apparatus here described has been devised for the purpose of studying small opaque objects under high magnifications by reflected light with uniform illumination on all sides. Since the source of light is distant, little heat is present and even this can be filtered out between source and mirror if desired. It has the added advantages of being simple, cheap, and durable.

An old compound microscope without condenser but with good lenses was used (Fig 1). A large circular opening was cut in the stage so that only the rim (A) remained. On top of this was cemented with Duco a piece of glass (B) the exact size of the stage and a small area was painted black (C) on the under side beneath the objective to shut out transmitted light. A large substage mirror (D) such as is used on binocular microscopes was substituted for the one of usual size. The concave metal reflector (Fig. 1 E and Fig. 2) was removed from a Strik-a-lite lamp, obtainable at many auto supply stores. In the edge of this on opposite sides shallow grooves one inch wide (F) were cut so that it would rest on the stage over a depression slide (G), the ends of which project through the grooves.

FIG. 2

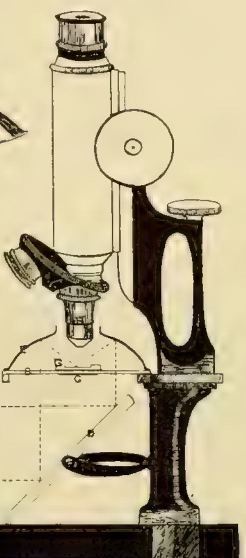


FIG. 1

The specimen is mounted in alcohol on the slide and placed over the painted black spot. The reflector is then dropped over the slide and the objective (H) focused down through the hole in its top. The mirror reflects a strong light from a distant source through the glass stage to the reflector which concentrates it on to the specimen uniformly from all sides. Minute opaque structures (antennal segments, ocelli, etc.) have been magnified to 450 diameters, studied, and camera lucida drawings made of them. This apparatus is in use in Room 3, Rockefeller Building and can be seen there by any one interested.

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole

Edited by Ware Cattell with the assistance of Margaret L. Goodson, Rita Guttman, Martin Bronfenbrenner, Elizabeth Jenkins, Margaret Mast and Annaleida S. van't Hoff Cattell.

THE COLLECTING NET IN 1933

The purpose of THE COLLECTING NET is to assemble material which is of especial interest to the workers in the biological institutions at Woods Hole. We want to record as fully as we can the research work and other activities of the members of the Marine Biological Laboratory, the Woods Hole Oceanographic Institution and the United States Bureau of Fisheries. In addition we want to seek relevant material outside of Woods Hole and to record local events of interest. The projected editorial contents of our magazine can be divided into four parts:

(1) Results of the scientific work reported during the summer at Woods Hole.

(2) Items reporting the activities of members of the scientific institutions in Woods Hole.

(3) World-wide news of the activities of institutions and individuals working in the field of biology.

(4) The more important local news.

THE COLLECTING NET is an independent publication. Its contents are based primarily on the three scientific institutions in Woods Hole, but it has no official connection with any one or them. We believe that there is not only a place, but a real need for an informal magazine of biology which is prepared to include constructive discussion on any topic of interest to those persons working in the biological sciences.

Sometimes material is submitted to an editor which he would like to print, but an editorial board makes its publication unwise or impossible. The fact that THE COLLECTING NET is responsible to no organization thus gives it a peculiar advantage over many publications in the field of science.

THE SCHOLARSHIP AWARDS

Last summer from many cources we obtained enough money for THE COLLECTING NET SCHOLARSHIP FUND to permit the award of six scholarships. The sum of one hundred dollars was assigned to each of the following five students in order to make it possible for them to work at Woods Hole during the present summer:

Name	Course
Iping Chao	Physiology
H. L. Eastlick	Embryology
J. J. Metzner	Protozoology
C. B. Havey	Zoology
Margaret Grierson	Zoology

The staff of the course in botany decided not to award a scholarship to one of its students.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 1	4:50	5:21
July 2	5:48	6:16
July 3	6:48	7:12
July 4	7:50	8:10
July 5	8:53	9:08
July 6	9:50	10:06
July 7	10:46	11:02
July 8	11:41	11:56
July 9	——	12:35
July 10	12:51	1:29

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

DIRECTORY FOR 1933

KEY

Laboratories		**Residence**	
Botany Building	Bot	Apartment	A
Brick Building	Br	Dormitory	D
Lecture Hall	L	Drew House	Dr
Main Room in Fisheries Laboratory	M	Fisheries Residence	F
Old Main Building	OM	Homestead	Ho
Rockefeller Bldg.	Rock	Hubbard	H
		Kahler	Ka
		Kidder	K
		Whitman	W

In the case of those individuals not living on laboratory property, the name of the landlord and the street are given. In the case of individuals living outside of Woods Hole, the place of residence is given in parentheses.

MARINE BIOLOGICAL LABORATORY

INVESTIGATORS

Adams, E. M. grad. biol. Cincinnati. Br. 8 Dr 2.

Amberson, W. R. prof. phys. Tennessee. Br 109. Quisset.

Anderson, R. S. res. assoc. phys. Princeton. Br 110. McInnis, Millfield.

Armstrong, Louise S. res. asst. anat. Cornell Med. L 22. D 315.

Armstrong, P. B. asst. prof. anat. Cornell Med. Br 318. D 315.

Atlas, M. asst. zool. Columbia. Br 314. Dr 5.

Baden, V. univ. scholar zool. Pennsylvania. Rock 6. D 210.

Bailey, R. J. instr. zool. George Wasington. OM Base. Stewart, School.

Baitsell, G. A. prof. biol. Yale. Br 323. Brooks.

Barron, E. S. G. asst. prof. biochem. Chicago. Br. 207. D 207.

Barth, L. G. instr. emb. Columbia. Br 111. Hubbard, East.

Beck, L. V. grad. asst. phys. Pittsburgh. Rock 7. Dr.

Beckwith, Cora J. prof. zool. Vassar. A 209.

Bell, H. P. prof. bot. Dalhousie. (Canada) Bot 23. D 216.

Bostian, C. H. asst. prof. zool. North Carolina State. Rock 3. Dr 5.

Bowen, R. E. asst. prof. biol. Long Island. Br 126. Howes, Water.

Bowling, Rachel instr. proto. Columbia. OM 21. A 307.

Boyden, Louise E. ed. sec. "Biol. Bul." Br 305. A 306.

Bridges, J. C. instr. zool. Morehouse. L 33. A 106.

Brinley, F. J. asst. prof. zool. North Dakota State. OM 38. D 201A.

Brooks, Matilda M. res. assoc. biol. California. Br 233. Gosnold.

Brooks, S. C. prof. zool. California. Br 233. Gosnold.

Brown, W. R. grad. biochem. Cincinnati. Br 342. D 109.

Budington, R. A. prof. zool. Oberlin. Br 218. Orchard.

Butt, C. tech. Princeton. Br 116. Dr.

Calkins, G. N. prof. proto. Columbia. Br 331. Buzzards Bay.

Cannan, R. K. prof. chem. N. Y. Univ. and Bellevue Hosp. Med. Br 310. Gardiner.

Cattell, W. assoc. ed. "Scientific Mo." Br 344. North.

Chambers, R. res. prof. biol. New York. Br 328. Gosnold.

Chao, I. grad. phys. Chicago. Br 315. D 106.

Chidester, F. E. prof. zool. West Virginia. Br 344. D 318.

Chute, A. L. Toronto Med. (Canada) OM. 9. Ka. 3.

Clark, Frances Sec. to dir. Lilly Res. Labs. Br. 328 B. Howe, Main.

Clark, Jean M. grad. Pennsylvania. Br 219. H 4.

Clowes, G. H. A. dir. Lilly Res. Labs. Br 328 B. Shore.

Coe, W. R. prof. biol. Yale. Br 323. A 201.

Coghill, G. E. mem. Wistar Inst. Anat. and Biol. Br 220. Veeder, West.

Coghill, Muriel grad. zool. Denison (Ohio) Br 220. Veeder, West.

Cohen, Rose C. grad. asst. zool. Cincinnati. L 29. H 6.

Conklin, E. G. prof. biol. Princeton. Br 321. High.

Corson, S. A. grad. res. asst. phys. Pennsylvania Med. Br 205. Young, West.

Costello, D. P. instr. zool. Pennsylvania. Br 217n. Elliot, Center.

Crampton, Clair B. instr. biol. Wesleyan. Br 210. D 110.

Croasdale, Hannah T. grad. bot. Pennsylvania. Bot. 22. W G.

Dan, K. grad. zool. Pennsylvania. Br 111. Clark, Millfield.

Day, Dorothy asst. prof. bot. Smith. Bot. A 205.

Denny, Martha grad. Radcliffe. Br 312. Kittila, Bar Neck.

Doyle, W. L. res. asst. phys. Hopkins. Br. 332. Dr 6.

Drumtra, Elizabeth asst. zool. Barnard. Br 314. K 3.

Eastlick, H. L. asst. zool. Washington (St. Louis) Br 217g. Grave, High.

Edwards, D. J. assoc. prof. phys. Cornell. Med. Br 214. Gosnold.

Edwards, T. I. instr. biol. Hopkins. L 21. Dr 7.

Engel, F. L. Dartmouth. Br 109. D 111.

Engel, G. L. Dartmouth. Br 309. D 111.

Finley, H. E. assoc. prof. biol. West Virginia State L 30. A 105.

Fisher, K. C. fel. Toronto (Canada). Br. 107. Ka 1.

Fowler, Virginia M. asst. bot. Barnard. Bot. K 3.

Fry, H. J. prof. biol. New York. OM Base. Purdum, Woods Hole.

Furtos, Norma C. fel. biol. Western Reserve. OM Base k. H 1.

Garrey, W. E. prof. phys. Vanderbilt Med. Br 215. Gardiner.

Gerard, R. W. assoc. prof. phys. Chicago. Om 3. D 303.

Goldforb. A. J. prof. biol. City N. Y. Br 122C. A 302.

Goodrich, H. B. prof. biol. Wesleyan. Br 210. D 110.

Grave, B. H. prof. zool. DePauw. Br 234. High.

Grave. C. prof. zool. Washington (St. Louis). Br 327. High.

Harnly, Marie L. asst. biol. New York. Br 1. A 102.

Harnly, M. H. asst. prof. biol. New York. Br 1. A 102.

Harvey, Ethel B. independ. invest. phys. Princeton. Br 116. Gosnold.

Harvey, E. N. Osborn prof. biol. Princeton. Br 116. Gosnold.

Hayes, F. R. assoc. prof. zool. Dalhousie (Canada). OM 45. D 213.

Heilbrunn, L. V. assoc. prof. gen. phys. Pennsylvania. Br 219. Spaeth, Whitman.

Hetherington, W. A. fel. zool. Hopkins. Br 329A. Gray, High.

Hibbard, Hope assoc. prof. zool. Oberlin. Br 218. K 12.

Hicks, F. J. grad. zool. Pittsburgh. Rock 7. Ka 21.

Hill, E. S. res. asst. physical chem. Rockefeller Inst. Br 207. D 218.

Hoadley, L. prof. zool. Harvard. Br 312. D 315b.

Hoijer, Dorothy J. Chicago Br 207. Neal, West.

Hollaender, A. Nat. Res. fel. biol. Wisconsin. Br 225. Sylvia, Buzzards Bay.

Hoppe, Ella N. res. biologist. N. Y. State Dept. Health. Br 122B. D 313.

Howe, H. E. ed. "Ind. and Eng. Chem." Br 203. Tinkham, West.

Hutner, S. H. grad. Cornell. OM Base. K 5.

Hyde, Ida H. emer. prof. phys. Kansas State. L 34. Nickerson, Millfield.

Irving, L. assoc. prof. phys. Toronto (Canada). Br. 107. A 202.

Jacobs, M. H. prof. gen. phys. Pennsylvania. Br 102. Sippewissett.

Jao, C. C. grad. bot. Michigan. Bot 26. Dr 9.

Jenkins, G. B. prof. anat. George Washington. OM 34. Clapp, Gardiner.

Johlin, J. M. assoc. prof. biochem. Vanderbilt Med. Br 206. Park.

Johnson, Arline C. grad. asst. zool. Oberlin. OM Base g. H C.

Jones, N. instr. sci. drawing. Swarthmore. Br 211. Hall, Main.

Kekwick. R. A. London fel. phys. Princeton. Br 127. Minot.

Keltch, Anna K. res. chem. Lilly Res. Labs. Br 319. McInnes, Millfield.

Kidder, G. W. tutor biol. City N. Y. Br 217. D 307.

Kirkpatrick, T. B. assoc. prof. physical education. Columbia. L 27. Nickerson, Millfield.

Korr, I. M. asst. instr. biol. Princeton. Br 110. Young, North.

Lancefield, D. E. assoc. prof. zool. Columbia. Br 333. Danchakoff, Gardiner.

Lancefield, Rebecca C. assoc. bact. Rockefeller Inst. Br. 208. Danchakoff, Gardiner.

Landowne, M. Harvard Med. Br. 108. Ka 24.

Liedke, Kathe B. grad. zool. Columbia. Br 314. Sylvia, Buzzards Bay.

Lillie, F. R. prof. zool. Chicago. Br 101. Gardiner.

Lillie, R. S. prof. gen. phys. Chicago. Br 326. Gardiner.

MacDougall, Mary S. prof. zool. Agnes Scott. A 208. L 28.

McLane, Kathryn E. instr. biol. Annapolis High School. phys. H 7.

Magruder, S. A. grad asst. zool. Cincinnati. L 29. Kittila, Bar Neck.

Manery, Jeanne F. res. asst. phys. Toronto (Canada). Br 107. H 2.

Marsland, D. A. asst. prof. biol. New York. Br 339. A 102.

Martin, E. A. chairman dept. biol. Brooklyn. OM 39. Newman, Prospect.

Mast, S. O. prof. zool. Hopkins. Br 332. Minot.

Mathews, A. P. prof. biochem. Cincinnati. Br 342. Buzzards Bay.

Mazia, D. grad. zool. Pennsylvania. Br 219. Ka 24.

Melampy, R. M. asst. animal nutrition. Cornell. OM Base. Dr 10.

Michaelis, L. mem. Rockefeller Inst. Br 207. D 209.

Miller, F. W. grad asst. zool. Pittsburgh. Rock 3. K 15.

Moreland, F. B. res. asst. biochem. Vanderbilt Med. Br. 206. Dr 5.

Morgan, T. H. prof. biol. Calif. Inst. Tech. Br 320. Buzzards Bay.

Morrill, C. V. assoc. prof. anat. Cornell Med. L 24. Cape Codder, (Falmouth).

Morris, S. grad. zool. Pennsylvania. Br 217m. D 305

Newton, Helen K. ms. ed. "Ind. and Eng. Chem." Br 203. Veeder, Millfield.

Nichol, Margaret A. grad. gen. Pennsylvania Col. for Women. Rock 3. W A.

Nonidez, J. F. asst. prof. anat. Cornell Med. Br 318. Whitman.

Novikoff, A. B. fel. biol. Columbia. Br 314. Dr 1.

Orbison, Agnes M. assoc. prof. biol. Elmira. OM 35. Nickerson, Millfield.

Packard, C. asst. prof. zool. Columbia Inst. Cancer Res. OM 2. North.

Palmer, A. H. instr. Univ. & Bellevue Hosp. Med. Br 310. Truslow, Gardiner.

Palmer, Elizabeth T. instr. chem. Vassar. Br 110 g. Truslow, Gardiner.

Parker, G. H. prof. zool. Harvard. Br 213. A 309.

Parpart, A. K. asst. prof. phys. Princeton. Br 205. Minot.

Pelluet, Dixie asst. prof. zool. Dalhousie (Canada). OM 45. D 103.

Prescott, G. W. asst. prof. biol. Albion. Bot 25. D 101.

Rex, R. O. instr. anat. Pennsylvania. Br 117. Conklin, High.

Richards, O. W. instr. biol. Yale. Br 8. A 303.

Richardson, Margaret S. Brearley School. Br 318. Hubbard, Center.

Root, W. S. assoc. prof. phys. Syracuse Med. Br 226. Oak, Park.

Rubenstein, B. B. asst. phys. Chicago. Br. 309. D 106.

Rugh, R. instr. zool. Hunter. Br 111. D 208.

Sauer, F. C. asst. prof. zool. Wichita. Br. 217. Thompson, Main.

Schechter, V. instr. invert. zool. City N. Y. OM 1. Dr 2.

Schweitzer, M. D. grad. zool. Br 333. McLeish, Millfield.

Scott, A. C. asst. zool. Columbia. Br. 314. Thompson, Main.

Sell, J. P. grad. asst. biol. Yale. Rock 7. Ka 21.

Shapiro, H. grad. biol. Princeton. Br 110. Edwards, School.

Shaw, I. res. asst. biol. Long Island. Br 126.

Shoup, C. S. asst. prof. biol. Vanderbilt. Br 206. D 301B.

Sichel, F. J. M. asst. instr. biol. New York. Br 339. Dr.

Sonneborn, T. M. res. assoc. zool. Hopkins. Br 330. D 204.

Specht, H. grad. phys. Hopkins. OM Base. Dr 6.

Speicher, B. R. grad. asst. zool. Pittsburgh. Rock 3. K 15.

Speidel, C. C. prof. anat. Virginia. Br 106. D 104.

Spek, J. prof. zool. Heidelberg (Germany). Br 223. D 316.

Stabler, R. M. instr. zool. Pennsylvania. OM 22. D 210.
Starkey, W. F. grad. zool. Pittsburgh. Rock 7. Ka 21.
Stewart, Dorothy R. asst. prof. biol. Skidmore. Br. 222. Stokey, Gardiner.
Stockard, C. R. prof. anat. Cornell Med. Br 317. Buzzards Bay.
Strong, O. S. prof. neur. and neuro-hist. Columbia. Br 8. Elliot, Center.
Stuart, Martha S. grad. genitics. Pennsylvania Col. for Women. Rock 3. W A.
Summers, F. M. tutor biol. City N. Y. Br 2171. A 104.
Sumwalt, Margaret asst. instr. phys. Pennsylvania. OM 3. W G.
Tashiro, S. prof. biochem. Cincinnati. Br 341. Park.
Taylor, G. W. Nat. Res. fel. phys. Princeton. Br 110. Cowey, School.
Taylor, W. R. prof. bot. Michigan. Bot 24. Whitman.
Wade, Lucille W. grad. biol. Hopkins Sch. Hygiene. Br 319. W. I.
Walker, P. A. fel. Harvard. Br 312. Thompson, Water.
Waterman, A. J. instr. biol. Brooklyn. OM 39. D.
Weisman, M. N. tutor biol. City N. Y. Br 217J. McLeish, Millfield.
Whiting, Anna R. prof. biol. Pennsylvania Col. for Women. Rock 3. Whitman.
Whiting, P. W. prof. zool. Pittsburgh. Rock 3. Whitman.
Wieman, H. L. prof. zool. Cincinnati. Br 334. D 311.
Willey, C. H. asst. prof. biol. New York. Br 232. A 301.
Wilson, E. B. DaCosta prof. emeritus zool. Columbia. Br 322. Buzzards Bay.
Wilson, Hildegard, N. asst. biochem. Univ. & Bellevue Hosp. Med. Br 310. Buzzards Bay.
Winsor, C. P. grad. phys. Harvard. L 21. (Cataumet).
Wolf, E. A. assoc. prof. zool. Pittsburgh. Rock 7. Elliot, Center.
Woodruff, L. L. prof. proto. Yale. Br 323. Gansett.
Young, Roger A. asst. prof. zool. Howard. Br 315. A 304.
Young, W. C. asst. prof. biol. Brown. OM 34. Kittila, North.
Zirkel, C. assoc. prof. bot. Pennsylvania. Bot 6. A 101.

STUDENTS

Albaum, H. G. fel. biol. Brooklyn. emb. Dr 1.
Alt, H. L. assoc. med. Northwestern Med. phys. D 217.
Amidon, Elaine W. Syracuse. bot. H8.
Armock, C. M. curator biol. Mus. Northern Arizona. emb. Crowell, Water.
Bates, M. N. grad. asst. zool. Oberlin. emb. Dr 2..
Bechtel, W. R. instr. biol. Edinburg High Sch. (Ohio) proto. Bosworth, North.
Bell, Ruth grad. asst. zool. Wellesley. emb. W B.
Bengel, W. Z. asst. anat. & emb. DePauw. emb. Ka 2.
Bosworth, M. W. asst. biol. Wesleyan. bot. K 6.
Botsford, E. Frances asst. prof. zool. Connecticut. phys. Stokey, Gardiner.
Boyer, D. C. grad. biol. Columbia. proto. Hilton, Millfield.
Campbell, Mildred F. teach. bot. Shortridge High Sch. (Indianapolis) bot. Hall, Main.
Chen, Y. grad. zool. Pennsylvania. emb. Elliot, Center.
Churney, L. grad. zool. Pennsylvania. emb. Ka 2.
Cunniff, Hilda S. bot. H 9.
Dennis, Nofa N. teach. biol. Portage County High Sch. (Ohio) proto. Clark, Millfield.
Derrickson, Mary B. phys. W D.
DeWolf, R. A. instr. zool. Rhode Island State. emb. (Hyannis.)
Foster, Edith F. Vassar. emb. Bosworth, North.
Glassmeyer, E. J. grad. biochem. Cincinnati. phys. D 109.
Godwin, M. C. asst. hist. & emb. Cornell. emb. K 7.
Greco, F. M. Hunter. emb. Kittila, Bar Neck.
Hamilton, Mary A. Elmira. emb. H 7.
Havey, C. B. Acadia (Canada) phys. Dr 1.
Hibbard, Jeanne Oberlin. phys. K 12.
Hirschfield, N. B. Univ. and Bellevue Hosp. Med. proto. McLeish, Millfield.
Hooper, Kathryn T. Wheaton. emb. Young, West.
Howell, C. D. grad. biol. Hopkins. phys. Dr.
Johnson, Edna L. assoc. prof. biol. Colorado. phys. A 305.
Kagan, B. M. Hopkins. Med. emb. K 6.
Kriete, F. M. DePauw. emb. K 9.
Kriete, F. M. DePauw. emb. Hilton, Main.
Lippman, R. W. Yale. emb. Hilton, Main.
McAuley, A. A. DePauw. emb. K 9.
McGehee, Elise grad. Newcomb. emb. Oak, Park.
MacIntosh, F. C. dem. pharm. Dalhousie (Canada) phys. Thompson, Water.
Mathews, R. S. Physicians & Surg., Columbia. phys. Dr 6.
Melampy, R. M. asst. anim. nutrition Cornell. phys. Dr 10.
Moreland, F. B. grad. res. asst. biochem. Vanderbilt Med. Phys. Dr 5.
Moser, F. grad. zool. Pennsylvania. emb. D 214.
Perkins, Irene T. grad. biol. Columbia. proto. A 204.
Poris, Ethel Hunter. bot. Kittila, Bar Neck.
Ramsey, Sally Elmira. bot. Bosworth, North.
Root, Charlotte M. Ohio Wesleyan. emb. H 6.
Rose, S. M. grad. asst. biol. Amherst. emb. K.
Ross, E. grad. physico-chem. biol. California. phys. Ka 2.
Rubidge, Karyl W. Vassar. emb. Bosworth, North.
Solandt, D. Y. res. fel. phys. Toronto (Canada) phys. D 107.
Solandt, O. M. res. asst. phys. Toronto (Canada) phys. D 107.
Spangler, Betty A. Wheaton. emb. Young, West.
Stricker, G. J. Yale. phys. Neal, West.
Stubbs, T. H. instr. biol. & chem. Emory. proto. Sylvia, Buzzards Bay.
Summers, F. M. grad. proto. Columbia. Br 217. A 104.
Sweadner, W. R. grad. asst. Pittsburgh. Rock 3. Dr 8.
Taylor, H. C. grad. asst. biol. Wesleyan. emb. K.
Tukey, Gertrude R. Smith. emb. H 7.
Turner, R. S. instr. biol. Dartmouth. emb. K.
Urban, J. instr. biol. Randolph High Sch. (Ohio) proto. Bosworth, North.
Vexler, D. E. grad. phys. Rutgers. phys. Ka 22.
Ward, Mary Wellesley. proto. Grinnell, West.
Wardwell, Judith S. grad. asst. zool. Wellesley. emb. W B.
Webster, M. Dorothy grad. bot. Dalhousie. (Canada) phys. Young, West.
Young, M. I. instr. biol. Junior Col. Augusta. proto Sylvia, Buzzards Bay.
Zinn, D. J. Harvard. emb. D 108.

CHEMICAL ROOM

Hours: 8:30 A. M.—12; 1:30—4:30 P. M. Sat. 8:30—12.

The Chemical Room supplies chemicals, glassware, clamps and support stands for use only at the Marine Biological Laboratory. Special apparatus, batteries, gauges and reducing valves for gas cylinders are issued at the Apparatus Room (Brick Bldg. room 216). Supplies that are to be used by investigators elsewhere, such as microscope slides, cover glasses, shell vials, etc., may be obtained at the Supply Department (Frame Bldg. back of Brick Bldg.) *Catalogs* of chemicals and apparatus *may be borrowed from the Apparatus Room.*

The following standardized solutions will be furnished in limited quantities during the season of 1933. Special solutions, buffers, and pH standards must be ordered at least *two days* before they are needed.

N 1.000:

Acetic acid	Sulphuric acid
Hydrochloric acid	Sodium Hydroxide

N 0.100:

Hydrochloric acid	Sodium hydroxide

Buffer mixtures:

Acetate pH 3.6-5.6	Borate pH 7.6-10.0
Phosphate pH 5.4-8.0	

Phosphate-citrate pH 2.2-8.0 (McIlvaine)

Indicators—Clark and Lubs series.

Color tube standards—on special order.

Compressed gases:

Carbon dioxide, hydrogen, nitrogen and oxygen must be ordered by the investigator from the person in charge at least *seven days* before they are needed.

For other standards inquire of the person in charge at the Chemical Room. Investigators expecting to use special solutions or standardized reagents after September 1 are requested to notify the Chemical Room, if possible, before August 15. The standardized reagents are not usually available before *June* 20 or after *September* 15.

Attention is invited to the *Formulae and Methods* published by the Chemical Room in THE COLLECTING NET (1930, 1932) for stain and chemical solubilities and the composition of solutions. Copies may be obtained at THE COLLECTING NET office.

Members of classes are not entitled to supplies other than those provided in their regular class work. Beginning investigators will receive supplies only on the authorization of the person under whom they are working for the season.

Certain common tools are available at the Chemical Room for temporary loan to investigators. In order that maximum use be made of these, it is necessary that they be returned within 24 hours. When needed by other investigators they are subject to recall and will then be collected by the janitors. Tools that are needed by the investigator for the entire season are to be obtained from the Apparatus room.

Supplies no longer needed will be collected if word is left at the Chemical Room.

Investigators are urged to co-operate with the Chemical Room by cleaning their glass-ware before returning it at the completion of their work. Investigators are requested to place their name and date of departure on the Chemical Room Bulletin Board so that their supplies may be returned promptly by the janitors.

When the investigator is continuing the same work in the same room during the next season his supplies may be retained in the room *only* if they are listed on a Kept Out card (furnished at the Chemical Room window) and the card left with the supplies. All supplies not so listed will be returned by the janitors. Should the investigator be unable to return the following summer the supplies will be returned to the Chemical Room stocks if they or the room is needed by other investigators.

Small amounts of special solutions will be kept during the winter for investigators in the Chemical Room on request. Supplies that may be injured by freezing should not be left in the wooden buildings. —OSCAR W. RICHARDS.

M. B. L. CLUB, PAST AND PRESENT

DR. L. V. HEILBRUN, *President.*

The M. B. L. Club is now nineteen years old. It came into being on July 16, 1914, following an organization meeting in the old lecture room of the Laboratory. At that meeting, Dr. Drew, then assistant director of the Laboratory, offered the use of what is now the Club building to any socially minded organization of laboratory workers.

The building itself was originally a yacht club and stood over the water. It was bought for the Laboratory through the generosity of Mr. Charles R. Crane, who apparently conceived of it as a social meeting place for men and women of the Laboratory. But when the M. B. L. first acquired possession of the building, it needed space for scientific activity more than for social meetings. This was before the days of brick buildings, and workers were badly crowded in the old wooden structures. In 1913, what was then called the Yacht Club Building was fitted out as a temporary laboratory. However, the old yacht club served the purposes of science only for a year. By 1914 the Crane Laboratory was completed and the Yacht Club Building could then be used for the purpose for which Mr. Crane had originally given it.

Like all clubs, the M. B. L. Club began life by drawing up a constitution. This states that the "object of the Club is to promote social intercourse among the scientific workers of the Woods Hole community and their friends." The active membership of the Club originally was limited to members of the M. B. L. and the U. S. Bureau of Fisheries, but in 1916 active membership was thrown open to all scientific workers of the Woods Hole community. Thus in 1916 there must have been some premonition of an Oceanographic Institution.

The first president of the Club was Dr. E. G. Spaulding, professor of philosophy at Princeton University, from which it might be supposed that philosophical discussion was fostered at Club meetings. Unfortunately, Club records are very brief and offer very little enlightenment on this point. Practically the sole information in the Club minutes is an expense account. From this record of expenses, it may be deduced that the only organized form of amusement or entertainment fostered by the club in its early years was tea drinking. Social teas were apparently held at regular intervals. There are various items in the ex pense account which may be interpreted as due to teas. Thus on Sept. 3, 1915, the Club paid to Mr. Sidney Peck $3.05 for "denatured alcohol for teas." If one were to interpret this item in post-prohibition ways of thought, biologists must have been a hardy lot in those days!

Magazines seem to have entered the Club about 1918. At any rate, before this year they are not noted in Club records. But on Aug. 2, 1918, "Dr. Clark mentioned that, in view of the fact that not more than three magazines had been donated to the Club, he had purchased magazines at news stands, costing, to date, $2.35." In recent years the Club has usually provided magazines for its members, and it has also made a practice of subscribing for *The New York Times.* Last year a small lending library was initiated, and this will be continued.

The war year, of 1918, appears to have initiated another Club activity. Possibly because of contact with sailors of the temporary naval base in Woods Hole, Club members became interested in dancing and a victrola was purchased. Prior to 1918, biologists at the Laboratory had scarcely indulged in dancing at all. There was, it is true, an annual waiter and waitress party at the Mess, and at these parties, following a moonlight sail on the Cayadetta, the one or two waiters who could dance tried out a few steps with a waitress or with Mrs. Coombs. And there were also dances at the Town Hall, but these were rarely attended by laboratory workers, most of whom could not dance. Perhaps because of their inability to dance, they were apt to scoff at the affairs in the Town Hall and to refer to them as "hog-wrastles." However, following 1918, dancing became an important Club activity, and many of the younger biologists learned various modern steps at the weekly Club dances. In 1929, the Club procured a loud speaker for the victrola and this has helped elevate the strains of dance records above the shuffling of feet. Sometimes, in periods of affluence, the club has felt rich enough to provide orchestras for its dances. Last year, the orchestral music was voted far preferable to the amplified victrola, and it is hoped to secure orchestras again for this season, at least for alternate weeks.

In the early years of the Club, the membership seems to have had a strong inclination toward song. Sunday nights wers usually chosen for "sings" and there was a great deal of enthusiasm and plenty of volume. Certain ballads came to be recognized as Laboratory favorites, and song books appeared with collections of these choice

(Continued on Page 24)

THE WOODS HOLE LOG

(Continued from Page 22)

Miss Katherine Goffin, daughter of Superintendent and Mrs. Robert A. Goffin, is home after graduating from Pembroke College of Brown University in Providence. Her major subject was biology. She was president of the Musical Club, member of class basketball and swimming teams, and of the German and Questions Clubs.

A 500-pound fish unknown even to Superintendent Goffin of the Bureau of Fisheries was caught Monday by Norman Benson in his nets. It was toothless and from Mr. Benson's description Mr. Goffin thought it belonged to the porpoise family. The fish was released, as Mr. Benson feared it would break his nets, and exact information is not available.

Rev. James Bancroft, pastor of the Church of the Messiah, is ill. His place was filled last Sunday by Rev. Henry Nott.

A special patrolman has been appointed by the Town of Falmouth for the summer here. Louis McLane of Falmouth is holding the position.

The first artificial ice plant in the vicinity has been installed by Sam Cahoon for the summer trade. It is an ammonia plant of 20 tons capacity.

The Washington String Quartet of Washington, D. C., will arrive at Woods Hole on July 6th for eight weeks of rehearsals and will give three or four concerts in Woods Hole and one in Waquoit. Frank J. Frost is sponsor of the quartet, which consists of Messrs. Schwartz, Breitenberg, Wargo and Hamer, all members of the Washington Symphony Orchestra, Washington, D. C.

Rev. Arthur W. Tansy, Fall River, will assist Father Hugh Gallagher at St. Joseph's Catholic Church this summer.

M. B. L, CLUB, PAST AND PRESENT

(Continued from Page 17)

numbers. In more recent years singing activity seems to have become centralized in the Choral Society under the leadership of Mr. Gorakoff. The Choral Society meets in the clubhouse on Tuesday and Friday evenings during the summer.

The activities of the M. B. L. Club have in the past extended beyond its clubhouse. Although it is not generally known, in recent years the Club has financed the maintenance of the raft at the more or less public bathing beach. In view of carpenters' prices at Woods Hole, this has been no mean expense. Use of the raft has not been limited to Club members, and accordingly laboratory workers, townspeople, and summer cottagers have all enjoyed it.

In view of the high cost of the raft, it is to some extent doubtful whether or not its maintenance will be continued by the Club. The decision must of course depend on the condition of the Club treasury and the speed with which members pay their dues.

As a matter of fact, the treasury is rather low, for last year the Club paid for a new coat of paint for its house, and it also paid an unusually large bill for the construction of a new raft in 1931. Our plans for the year depend on finances. We hope to expand the small lending library, and we expect to provide at least the usual number of magazines. It has been suggested that a ping pong table might be appreciated on rainy evenings. If such a table were desired, it could be procured at no very great expense.

One of the primary objects of the Club is to make life pleasant for the Woods Hole summer colony. It can be a real help to laboratory workers and their families. Often men and women who come to Woods Hole feel strange here. The Club can help these newcomers to become better acquainted. This year it proposes to do this in two ways. In the first place, on July 1 it will hold its annual opening reception, the so-called mixer. This part of the program is for all biologists and their families, whether they are Club members or not. Every one is urged to come. As at previous mixers, there will be dancing, but in view of the fact that many do not care to dance, the orchestra will not begin to play before 10:30.

In addition to the mixer, it is also planned to provide a second means of getting people acquainted. Each evening during the month of July, we plan to have a host or hostess present at the Club. It will be the duty of such host or hostess to introduce members to each other. If our plan succeeds, it should be possible for newcomers in our community to meet congenial companions.

It is the hope of the officers of the M. G. L. Club that during the summer of 1933 the Club may serve a real purpose in making life pleasanter for the entire community. With proper cooperation from Club members, we shall no doubt succeed in our aim.

THE COLLECTING NET

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HEMOGLOBIN-RINGER: A NEW MAMMALIAN PERFUSION MEDIUM

DR. WILLIAM R. AMBERSON
Professor of Physiology, University of Tennessee

Several years ago, in perfusion work with the salivary glands of the cat, Professor Rudolf Höber and I encountered serious difficulties with several of the fluids ordinarily used in such work. Both whole defibrinated blood and blood serum gave marked vaso-constriction which speedily terminated the perfusion flow, while oxygenated Ringer of the usual type, without colloids, gave edema, and a spontaneous secretion of saliva which was definitely pathological in character.

In an attempt to secure a perfusion fluid which would avoid such difficulties, we finally decided to add a certain amount of hemolysed beef red blood cells to our Ringer solutions, hoping to find that hemoglobin in solution would not only transport the necessary oxygen, but would furnish a colloidal osmotic pressure which would regulate the fluid balance and prevent edema. We found that these expectations were (*Continued on Page* 32)

M. B. L. Calendar

MONDAY, JULY 10, 8:00 P. M.

Seminar: Dr. A. C. Redfield: The concentration of organic derivatives in sea water, in relation to the chemical composition of plankton.

Dr. George L. Clarke: Diurnal migration of plankton in the Gulf of Maine and its correlation with changes in submarine irradiation.

Dr. Selman A. Waksman and Miss Cornelia L. Carey: The role of bacteria in the formation of nitrate in the sea.

TUESDAY, JULY 11, 8:00 P. M.

(Continued on page 39)

THE BIOLOGICAL LABORATORY AT COLD SPRING HARBOR

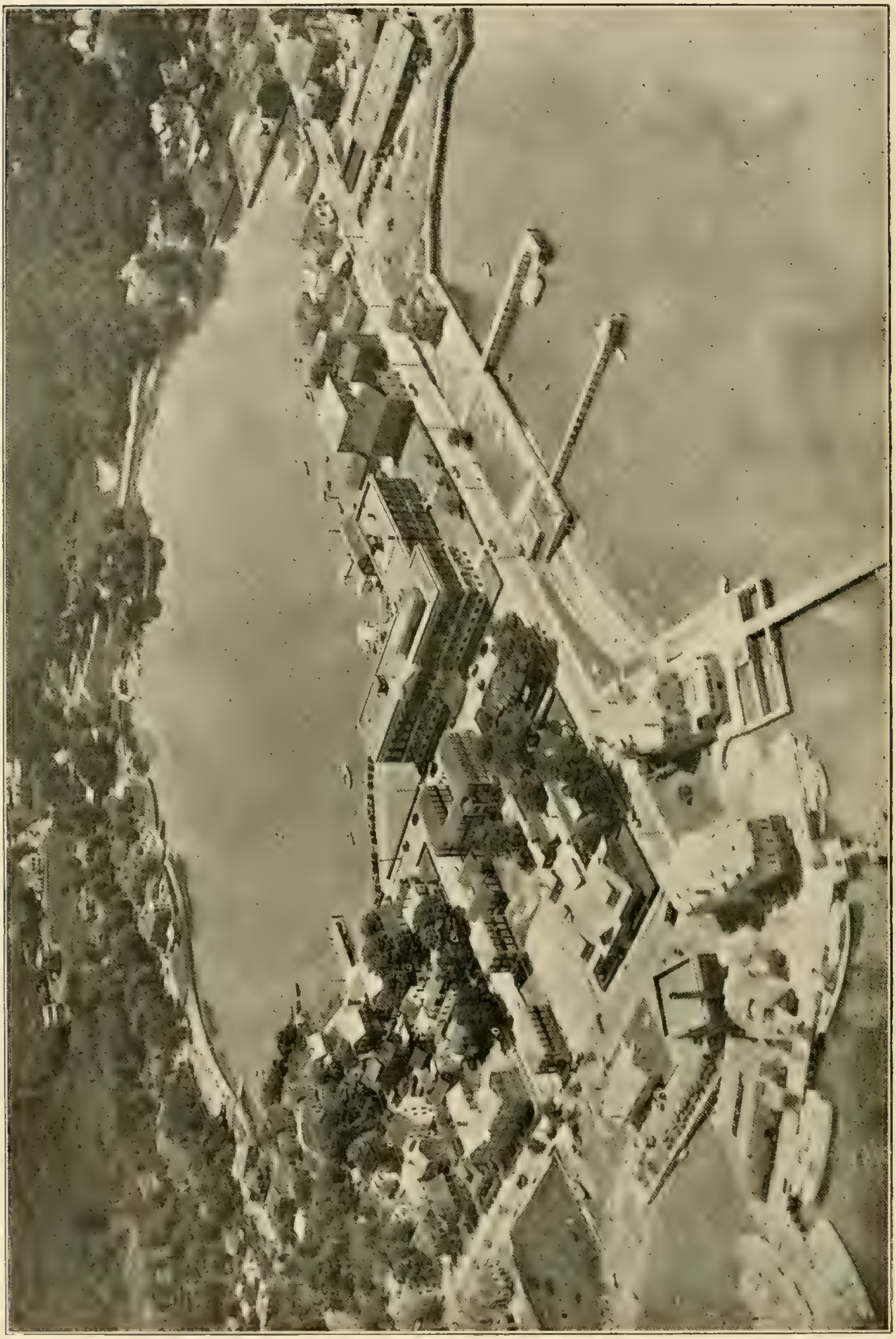
DR. REGINALD G. HARRIS
Director of the Laboratory

Causing plans to fade out slowly and to be replaced by realization and accomplishment is one of the most difficult tasks of a laboratory, particularly in times of financial depression. And yet that is precisely what the Laboratory at Cold Spring Harbor has been trying to do.

In outlining our plans last year in THE COLLECTING NET we said, "- - - the development of an institute in which biologists, physiologists, chemists, physicists and mathematicians will cooperate in the further opening and beneficial use of the vast territory of quantitative biology, is the direction in which our hopes are for the future in respect to the all-year work of the Biological Laboratory."

The Laboratory is now beginning to test this plan as a part of its summer work. Each summer a group of mathematicians, physicists, chemists and biologists, actively interested in a given aspect of quantitative biology, (*Continued on page* 31)

TABLE OF CONTENTS



THE BIOLOGICAL LABORATORY AT COLD SPRING HARBOR

(Continued from Page 29)

or in methods and theories applicable to it, will be invited to carry on their work, to give lectures and to take part in symposia at the Laboratory.

The groups in residence at the Laboratory will necessarily be relatively small, but members of the group will be chosen with the aim that every important aspect of a given subject be adequately represented from the physical and chemical, as well as from the biological point of view.

Just what we have in mind is no doubt most easily explained by a description of what we are doing this year. The interest of the group chosen this summer will be centered on "the electrical potential difference at interfaces and its bearing upon biological phenomena." Members of the group cover various aspects, from theories of physics to application to medicine. Thus Dr. Hans Muller, of the Department of Physics of the Massachusetts Institute of Technology, represents aspects concerned with physical theories, and will lecture upon theories, such as those of the diffuse double layer, of cataphoretic migration, of coagulation.

Dr. Stuart Mudd, of the Department of Bacteriology of the School of Medicine of the University of Pennsylvania, will represent aspects concerned with the application to medicine. The titles of his lectures, "Agglutination", and "Phagocytosis" are indicative of his role in the group.

Other members of the group in residence represent various experimental aspects of the subject, and may include theory and application to medicine. Dr. Harold Abramson is concerned primarily with cataphoresis; Dr. David R. Briggs with electrosmosis, and streaming potentials; Dr. Barnett Cohen, with oxidation-reduction potentials; Dr. Kenneth S. Cole, with electrical conductance and polarization; Dr. Eric Ponder, with physical properties of red corpuscles.

Other workers have been invited to take part in the symposia in connection with the group meeting. These men include, in addition to Dr. Hugo Fricke, who is in charge of our laboratory for biophysics, Dr. Chambers, Dr. Gasser, Dr. MacInnes, Dr. Michaelis, Dr. Osterhout and Dr. Van Slyke. Their part in the program is indicated in the list of lectures and symposia which is published elsewhere in THE COLLECTING NET.

This, then, is the putting into practice, this time in respect to the Laboratory's summer work, of the second major step in our policy of fostering a closer relationship between biology and the basic sciences. The first step was the establishment in 1928 of our all-year laboratory for biophysics. Incidentally, the fact that the laboratory for biophysics is functioning here throughout the year has been of very great practical aid in the inauguration of our plan for group work and conference during the summer.

The description of the plan, as it is operating this summer, is the best indication I can give of the way in which it will be developed in subsequent summers. The subject will, of course, change from year to year, but the fundamental aim and the type of men chosen to aid in the realization of that aim, will, in all probability, remain similar to those of this year.

It is expected that many advantages will be secured through the operation of the plan. Outstanding among these is the value of the meetings to the men who form the group. Support of this assertion is to be found in the fact that, without exception, every man who was asked to become a member of the group, and who planned to be in this country during the summer, accepted.

Another advantage lies in the fact that the presence of such a group at Cold Spring Harbor each summer will aid the Laboratory in its primary aim, namely that of being of as much service as it can be in advancing biology. Everyone at the Marine Biological Laboratory at Woods Hole is wholly familiar with the desire of the founders of that Laboratory and of those who established the Laboratory at Cold Spring Harbor, i. e., that these stations should not only provide opportunities for summer work, but that they should be centers of growth and dissemination of new methods and ideas in biology. In my opinion, much of the ultimate value of the summer work of both institutions will always remain in the fulfillment of the basic desire which gave them birth. The opportunity to foster the development of promising methods and ideas is greater now than when these laboratories were founded, and the number of relatively isolated colleges and universities in this country which can be benefited by active, conscious effort, on the part of summer laboratories, to stimulate interest in modern methods of research, is much larger than it was in the last quarter of the nineteenth century.

The Laboratory's plan to choose each year a group interested in an aspect of quantitative biology different from that of the year before, insures against our giving undue stress to any one part of modern biology, and at the same time brings a partially new mental environment to workers who, for one reason or another, prefer to spend every summer at the same laboratory.

Parenthetically, there is, of course, the further consideration that the presence of a definite program of work within a summer laboratory seems to stimulate all of those in residence, and tends to eliminate the job-hunters and vacation-seekers who curtail the work of any institution at which

THE AERIAL VIEW OF WOODS HOLE ON THE OPPOSITE PAGE WAS TAKEN FOR THE COLLECTING NET BY A UNITED STATES ARMY AIR CORPS PHOTOGRAPHER

they congregate. In this connection we have found at Cold Spring Harbor that the all-year work of the Laboratory, with its definite program, provides a marked stimulus to summer workers.

Finally, the Laboratory hopes to make available to all workers interested in the aspects which will be subjects of its group meetings, the tangible results of these meetings. This will be done through the lectures and symposia, which assume, therefore, a very great importance. These lectures and symposia should not be confused with the evening lectures and seminars which have been, and will continue to be, a part of the Laboratory's summer work. The lectures and symposia connected with the group meetings will be given during the day, beginning at ten o'clock in the morning. Ample time will be allowed for discussion. Indeed lecturers have been asked to give special consideration to theoretical and controversial aspects of the subject, in order that discussion may be significant and creative. Furthermore, in general, students and others not competent to take an active part in discussion will not be invited to attend the lectures and symposia. Attendance is not restricted, however, to scientists in residence at the Laboratory. Moreover, it is planned to publish the lectures, together with essential parts of the discussion, as a monograph, in order to make results of the meetings generally available.

A small number of graduate students will be given the opportunity of working with members of the group who are in residence at the Laboratory and who will further give special lectures for students from time to time, notably for those in the course in general physiology.

The whole plan is obviously not the creation of any one man. All of the members of this year's group have been helpful in offering suggestions, while Dr. Osterhout has been called upon frequently to give counsel.

The other summer work of the Laboratory remains fundamentally unchanged. The number of students is at the reduced level which has been maintained during the past few years, namely about thirty-five. Prof. W. W. Swingle of Princeton University continues in charge of the course in Surgical Methods in Experimental Biology; Prof. S. I. Kornhauser of the University of Louisville Medical School again leads the course in Field Zoology; Prof. I. R. Taylor, of Brown University, that of General Physiology, and Prof. H. S. Conard, of Grinnell College, that of Plant Sociology; while Prof. Bert Cunningham of Duke University will give a series of lectures on the endocrine system. Dr. A. J. Grout will again have a limited number of bryologists working under his direction at his personal laboratory at Newfane, Vermont.

Provisions are made, as before, for independent investigation, and for young research workers to use the facilities of the Laboratory.

The inauguration of the group work will not effect detrimentally the opportunities which have been traditionally extended to the "lone eagle" biologist. Indeed the present plan has been brought into existence not to replace the previous work of the Laboratory, but rather to strengthen it and to aid it in fulfilling its destiny *vis a vis* with present and future advance in biology.

HEMOGLOBIN-RINGER: A NEW MAMMALIAN PERFUSION MEDIUM

(Continued from Page 29)

justified in our further studies, and we were enabled to carry out a series of experiments on the permeability of the salivary glands for various organic non-electrolytes, which have been described elsewhere.

More recently my colleagues at Memphis and I have been interested to see whether "hemoglobin-Ringer" might be adapted to other mammalian problems. We were particularly concerned to know whether this fluid might be used to replace the blood in the whole animal, and are able to report that this may be done, with a continuation of most, if not all, vital functions, for a period of hours after the removal of the normal blood.

The adequacy of the medium as a substitute for blood can be demonstrated in a variety of ways. We consider that our determinations of the oxygen consumption furnish the most convincing proof. Using an apparatus designed and described by Dr. Arthur G. Mulder we have been able to show that the oxygen consumption of the whole animal under veronal anesthesia continues with practically no change for several hours after the replacement of the normal blood by hemoglobin-Ringer. The hemoglobin in solution is able to fulfill its respiratory rôle much as when it was enclosed within the red blood cells.

Another convincing demonstration of the adequacy of the medium to support all vital functions is given by the return of consciousness in cats from which the blood has been removed under ether anesthesia. Such animals come out from ether in a manner hardly to be distinguished from the behavior of a normal animal under similar circumstances. The respiration, however, is more rapid and deeper than normal, and respiratory disturbances of several types are observed during the succeeding hours. In spite of this abnormality, the animals are able to carry out a variety of activities. They are able to walk, run, see and hear. They are able to jump to the floor from a considerable height, judge distances correctly, and

make their way about in a normal manner. All postural and equilibratory reflexes are functional. When dropped upside down they land on their feet. They recognize a dog as an enemy and will attack it with swift blows from their forepaws. While swift movements are possible they tire quickly and spend much of the time in sleep. They may be readily aroused and show a high degree of activity, only to lapse rather suddenly into sleep.

Such behavior is almost certainly connected with the initial low oxygen capacity of the hemoglobin-Ringer, and with the progressive loss of the hemoglobin from the blood stream as the experiment continues. Death finally comes to these animals, not because the hemoglobin in solution is unable to carry out its respiratory function, but because it leaves the blood stream, appearing particularly in the urine. It also may appear in the feces, and is to some extent taken up by cells of the reticulo-endothelial system.

The conclusion that death results from asphyxia when the hemoglobin concentration in the blood has fallen below a critical value is strengthened by our observation that an increase in the amount of hemoglobin added to our solutions prolongs the life of the animals. In our earlier experiments we used the hemoglobin from 170 cc. of beef cells in each liter of solution. Our animals survived for five or six hours. More recently we have raised the concentration of hemoglobin about 50%, using 250 cc. of cells per liter. Our animals now live from ten to fifteen hours. We expect to make further increases in hemoglobin content in an attempt to get longer survivals, and, possibly, recovery, if the production of new red blood corpuscles can occur rapidly enough to replace the hemoglobin which is leaving the body.

In its present form, with 250 cc. of red cells used per liter, our solution has an oxygen capacity of 11 to 12 volumes per cent. Through the courtesy of Dr. Herbert Wells, of Vanderbilt, we have recently measured the colloidal osmotic pressure of the solution, by a modified Krogh method, and secured a value of 26 mm. mercury in a single determination. This value is quite within the range for the normal colloidal osmotic pressure of mammalian blood. One of the main considerations leading us to use the solution in the first place was the hope that the hemoglobin might supply a colloidal osmotic pressure which would regulate the water balance. This hope has been fully justified. At autopsy all tissues appear normal in size and color; edema is definitely not present. It might be added that, while hemoglobin passes into the urine with great ease, it does not leave the blood stream in most tissues, so is able in our solution to exert an osmotic pressure approximating that of the normal plasma colloids.

Aside from the theoretical interest of our observations, we believe that this solution may be employed in the study of many problems involving the blood and circulation. For instance, blood volume may be easily and accurately determined, as in the Welcher method, with the assurance that all blood cells have been removed from the body. We have begun a study of the rate of replacement of various blood constituents. The leucocytes are rapidly replaced. Fibrinogen also returns rapidly, and has reached about 50 per cent. of the normal value in ten hours after the conclusion of the bleeding. Fluid withdrawn at this time will form a clot, the hemoglobin-Ringer setting into a clear red jelly.

We are led by these experiments to the conclusion that a very major function of the vertebrate red blood corpuscles is to hold hemoglobin within membranes impermeable to it, so that it cannot leave the blood stream. In other respects, hemoglobin appears to be able to carry out its respiratory rôle in solution much as it does within the red cells, sustaining every vital function, even the more complicated activities of conscious life.

COMMENTS ON THE PAPER BY DR. AMBERSON

DR. AUGUST KROGH

Professor of Zoophysiology, University of Copenhagen, Denmark

The hemoglobin-Ringer of Dr. Amberson will undoubtedly prove a very valuable addition to the perfusion fluids already available, all of which have certain drawbacks. It is an interesting fact that an animal can retain or regain consciousness when supplied with this fluid instead of blood, and it is especially interesting because Barcroft has recently shown that when the environmental balance of the tissues is upset, the first organ to suffer is usually the brain and loss of consciousness is one of the first symptons.

Even this perfusion fluid, however, is not in all respects *the* ideal one. It seems to be quite toxic to the renal glomeruli, since the normal hemoglobin molecule is certainly large enough to be kept back by the normal glomerular epithelium. The potassium of the red cells must make the hemoglobin-Ringer comparatively high in potassium unless this is allowed for in the Ringer by leaving out some potassium or by adding a corresponding surplus of calcium. I expect that the increase in viscosity will set a limit to the quantity of red cells which can be used to advantage.

Courses at the Marine Biological Laboratory

THE PHYSIOLOGY COURSE

DR. R. W. GERARD
Director of the Course, Associate Professor of Physiology, University of Chicago

The physiology course got well under way this summer on June 20 and has already succeeded in shocking most of the community with the aid of a large electric ray. The eighteen students have a considerable choice of techniques and materials to study under the direction of the staff members. Besides the regular staff, including Drs. Amberson, Gerard, Irving, Michaelis, and Sumwalt, we are fortunate in having Drs. Chambers and Lucké contribute some laboratory instruction. Mr. A. L. Chute is continuing his able assistance with the apparatus.

As in the past, the regular six weeks course is divided into three two-week periods. During the first two of these the various staff members offer selected experiments, largely along the line of their own research interests, which enable the students to acquire the appropriate experimental techniques and apply them to the study of familiar and novel materials.

In the first period, work is being offered by Dr. Amberson on bio-electric phenomena, such as action and injury potentials, and on electrokinetic behavior. He is also attempting for the first time a section on the physiology of secretion, using kidney and salivary glands. Dr. Irving is offering the Haldane and Van Slyke techniques in the study of carbon dioxide content, buffering power, acidity and the like, in tissues and biological fluids. Dr. Michaelis is taking many of the students into his own laboratory to study electrometric and other means of determining pH and oxidation-reduction potentials.

During the second two week period, Dr. Gerard will introduce the Warburg and other techniques for studying cell respiration. Dr. Sumwalt and Dr. Lucké will offer studies on the permeability of cells to water and dissolved materials, as influenced by temperature acidity, and the like. Dr. Chambers will supervise micro-dissection work done during both periods.

The last two week unit constitutes essentially an introduction to research, the students being encouraged to select some small problem along the lines of their previous experience and work at it intensively under the guidance of a staff member. A few students who make a promising start may carry on their research throughout the summer.

A lecture will be given at nine o'clock each weekday morning; at first by the regular staff members and later by other members of the scientific community whose research interests enable them to speak with authority on various subjects of significance to the course. These lectures, as in the past, are open to all interested members of the institution. Notices will be posted at intervals announcing current lectures.

MORPHOLOGY AND TAXONOMY OF THE ALGAE

DR. WILLIAM RANDOLPH TAYLOR
Director of the Course; Professor of Botany, University of Michigan

Cut off from popular observation and knowledge by submersion in an aquatic environment, the algae have long been a familiar and closely studied group of plants for but a small number of biologists. Their importance to the population of the waters is quite similar to that of land plants in the biological population of the higher ground, for in both cases the presence of animals is limited by the presence of the plants and by their activities in synthesizing carbohydrate and simple nitrogenous food substances. The importance of the algae extends from bodies of water to the land, for culturing of soil shows a very considerable and active algal flora in the upper layers even when not so superficial as to be visable to the unaided eye.

The ecological studies involving algal populations have too often been taxonomically inaccurate, and have dealt vaguely with the presence of genera lone, even when a genus ranged widely in adaptability. Likewise in local listing of algae, far too little care has been exercised in securing a critical determination. In the algae course at Woods Hole an introduction to the group is first made by a consideration of the general classification of these plants. With this to preserve order in ideas, the morphology and life history is considered in detail. At the present time active study of life-histories and sexual differentiation both in America and abroad has suggested many wide-reaching revisions of classification, which, even when incomplete and tentative, must be con-

sidered. It is possible also to call attention to the more marked physiological peculiarities of the various algal types. Every effort is made to secure living material to represent each family; in the case of the strictly tropical groups, a collection of preserved specimens of the more important genera is available.

In order that the students may have experience in the field, trips are taken at frequent intervals to collect material in a manner which would be suitable to an initial or exploratory survey of the flora of the area. On returning, the material is searched and the genera present determined, this involving the use of introductory manuals. From those students who show sufficient critical ability, more accurate identifications are encouraged, involving the use of original bibliographic sources. The smaller number of local marine algae enables all students to become familiar with these species in a detailed way.

The excellent library facilities of the Marine Biological Laboratory give especial encouragement to students wishing to begin algal studies. With its aid, familiarity may be secured with all of the important publications needed to begin taxonomic or morphological studies; for the student with a problem already begun, no better opportunity could be secured to do the detailed library work which is the necessary accompaniment and often the most time-consuming part of modern critical research. Students with major interests in the physiological or biochemical phases of life will find the library even more complete in their lines and may pursue their work with the aid of the botanical and physiological research staffs.

THE COURSE IN PROTOZOOLOGY

Dr. Gary N. Calkins

Director of the Course, Professor of Protozoology, Columbia University

The course in protozoology is designed for graduate students wishing to begin research in protozoology or in other fields requiring a knowledge of unicellular animals, free-living or parasitic.

The lectures cover the principles of general biology; the morphology of the different classes of protozoa; the parasitic protozoa and the diseases caused by them; experimental protozoology and the theories of growth and cell division, of fertilization, of age and reorganization, of heredity and variation.

The laboratory work gives an opportunity for the collection of material from natural sources; the study and identification of living organisms representing the main groups of the protozoa (seventy-five drawings) classified through genera, including ten classified through species, are required); the cultivation of protozoa in artificial media; the determination of the pH of media and of water collected from ponds around Woods Hole.

Opportunity is also given for special cytological studies of the protozoa. For this purpose, permanent preparations (ten) are required. In addition to the ordinary techniques, osmic and silver impregnation, the Feulgen nucleal reaction, and the chondriosome methods are employed. Fresh material stained by vital dyes is used to supplement fixed material when the identification of cytoplasmic elements is desired.

EMBRYOLOGY COURSE

Dr. H. B. Goodrich

Director of the Course; Professor of Biology, Wesleyan University

The embryology course opened on June 22. The chief objects of the course are to give opportunities for studying the development of living eggs and embryos and to present various fields of embryological research. Fertilization cleavage and stages of development are studied in representation of different phyla. It is not possible to arrange material in logical order, because the schedule is dependent upon the breeding seasons rather than on zoological classification. Fish are studied first as their spawning season comes early. The eggs of the annelid Nereis can only be obtained between full moon and the new moon.

The tentative schedule is given below. From time to time special lectures are given by various investigators.

MECHANICS OF MATING IN LIMULUS

Charles Marc Pomerat
Department of Biology, Clark University

Quantitative studies of mating behavior tend to support the view that the mating phenomenon does not rest upon chance pairing but that a considerable degree of assortative selection can be demonstrated. Mating of like with like is frequently designated as homogamy. The intensity of this tendency usually is measured by correlation coefficients derived from various physical characteristics of the species under consideration.

Of the various possibilities (mechanical, chemical, psychic, relative activity and physical vigor), the simplest explanation for the existence of homogamy in the majority of invertebrates studied thus far appears to rest upon purely mechanical factors involved in the mating act. On the basis of such an assumption, high homogamy correlations may be expected for organisms whose mating involves exact juxtaposition of more than one point of contact. Moreover, the presence of a more or less inflexible cuticula or exoskeleton will tend to intensify the mechanical limitations.

Such a theory is of value in an attempt to explain the findings already reported and to suggest possible checks on the theory itself. Conjugation in the protozoans, *Paramoecium* and *Chilodon;* hermaphroditism in *Chromodoris*, and the mechanical restrictions observed in mating gammarids suggests that the high correlations obtained for homogamy in these organisms may be accounted for on the basis that the mechanical precision involved does not permit coupling of individuals of widely divergent sizes. The low values obtained for the Japanese beetle, *Popillia japonica* Newm., do not tend to uphold this view unless it can be considered that a greater degree of adjustment at the contact points is possible than might *a priori* be expected.

Copulation in *Limulus polyphemus* L. involves amplexus but without intromission, so that only one pair of contact points are involved, i. e., clasping without genital contact. Owing to this fact *Limulus* serves as an excellent contrast to the organisms previously studied. In addition, the large size of this organism considerably reduces the technical difficulties usually involved in obtaining biometric data on invertebrates. With the aid of a Collecting Net scholarship the writer studied the mating behavior of *Limulus* at Woods Hole during the summer of 1932. Approximately 1400 measurements were taken on 100 pairs of mated *Limuli*. A mean value of 0.10 ± 0.06 was obtained from 49 direct and cross correlation coefficients for the seven characters studied. It was concluded that homogamy as regards size in Limulus is practically non-existent. A full account of this study has been published in the *Biological Bulletin,* LXIV, 2; 243-252, April 1933.

In addition to the increased range of choice afforded by the single-point type of clasping contact, a further analysis of the mechanics of mating in *Limulus* suggests that clasping in this animal depends upon a device which probably permits copulation between any male and female.

The second pair of cephalothoracic appendages of the adult female *Limulus* is like the third, fourth and fifth in having a chela—that is to say the penultimate sclerite is produced so as to form with the last sclerite a pair of nippers. In the male this is not the case, the second pair being thicker and heavier than in the female and the penultimate joint not prolonged. It was observed long ago that the form of this appendage in *Limulus polyphemus* closely resembles the appendages of Arachnids such as *Thelyphonus,* though not of *Scorpio.* By means of this hook-like modified pincers the male attaches himself to the hinder half of the carapax of the female and trails along in shallow water. In all adult males observed the jaw of the claw has been found to be wider than the thickest carapax rim ever observed in the female. On this basis the male may clasp any female.

It has been concluded that there is reason to believe that there exists very little mechanical restriction in the coupling of *Limuli* and that the range of choice is therefore markedly increased and the coefficient of homogamy proportionately lowered. The findings in *Limulus polyphemus* are in keeping with the notion that mating in certain invertebrates rests at least in part upon a mechanical process.

FURTHER STUDIES ON THE CONTROL OF ELASMOBRANCH MELANOPHORES

Helen M. Lundstrom and Philip Bard

Our previous work (Biol. Bull., 1932, 62, 1-9) has shown that a melanophore-expanding substance is continually being secreted by the posterior lobe of the dogfish pituitary. Removal of this structure is followed by extreme cutaneous pallor, which is permanent. This change is uninfluenced by the degree of illumination. Injection of post-pituitary extracts into such pale fish restores the normal dark coloration.

This summer we have investigated the mechan-

ism governing the changes in pigmentation which occur with changes in illumination. When dogfish with normal eyes are placed in a brightly illuminated tank they slowly become paler. This pallor is never as great as that produced by hypophysectomy, and its development requires several hours. When fish, which have been made pale in this way, are restored to a dark tank they darken rapidly—within thirty minutes. The same rapid darkening to a maximum (complete expansion of melanophores) takes place when a pale illuminated fish remaining in the illuminated tank has been subjected to any one of the following procedures: (a) covering the eyes with a light-proof material, (b) section of the optic nerves, (c) injury of the hypothalamus at any point between optic chiasm and the posterior hypophysis. These same procedures carried out before exposure to light prevented paling. On the other hand, section of the spinal cord at any level or complete transection of the brain just behind the pituitary (decerebration) did not produce darkening of light individuals, and these operations left the animals able quite normally to alter their shade with changes in illumination.

From these results we conclude that optic stimulation influences the secretion of melanophore-expanding substance from the hypophysis by a nervous process whose path involves optic nerves and hypothalamus. The final segment in this path must consist of fibers passing to the pituitary from the hypothalamic region.

In these experiments temperature, an important factor in the pigmentary responses of amphibia, was controlled. The variation in temperature between the illuminated and control tanks was never more than one degree Centigrade. Similarly care was taken to maintain the same degree of aeration in the two tanks.

When a large part of the body of a fish was covered, leaving only the anterior end exposed, the entire fish paled when illuminated, but it was observed that the covered area was very slightly darker than the exposed region. The difference was only just perceptible. Whatever the explanation of the phenomenon may be, it seems quite correct to state that variations in the degree of cutaneous pigmentation of the dogfish are almost wholly due to variations in pituitary secretion, and that these variations are induced by changes in illumination of the eyes acting through nervous channels on the secretory mechanism.

THE "ATLANTIS" AND ITS OCEANOGRAPHIC WORK

Columbus O. Iselin, 2nd
Reseach Associate in Physical Oceanography

Since last November the "Atlantis" has cruised about 10,000 miles and has occupied some 225 stations. She returned to Woods Hole at the end of May. The time in port until June 19 was utilized for a general spring overhaul so that she could be made shipshape before starting on the summer program. The first part of this is involving four short cruises to the Gulf of Maine, and then a longer trip northward is planned to begin in August. The summer program can be outlined briefly as follows.

June 19-*June* 28. First half of the chemical survey of the Gulf of Maine; U. S. Bureau of Fisheries haddock spawning investigations. Mr. Herrington was in charge.

June 29-*July* 8. Second half of the Gulf of Maine investigations and bacteriological samples for Dr. Waksman. Dr. Redfield is in charge.

July 10-*July* 16. Light intensity and the vertical migration of plankton. Dr. Clarke will be in charge.

July 20-*July* 25. Bottom samples from the deep water south of Woods Hole. Mr. Iselin will be in charge.

Aug. 12-*Sept.* 15. An investigation of the formation of bottom water in the North Atlantic. The deep layers will be followed northward until the region where they approach the surface is reached. Dr. Redfield will be in charge.

The main feature of the work done last winter was a thorough survey of the whole Caribbean Sea. In this program the Woods Hole Oceanographic Institution cooperated with the Bingham Oceanographic Foundation at Yale, which had made a good beginning the year before in the Gulf of Mexico. Dr. A. E. Parr was the scientist in charge.

Besides the routine temperature, salinity and oxygen observations, the majority of water samples were analyzed for nitrates, phosphates and pH. Since echo sounding equipment was installed on the "Atlantis" last autumn, hourly soundings were taken during the course of the trip. This should add greatly to our knowledge of the bottom contours along the many sections across the Caribbean Sea as well as in the passages between the islands. Another innovation was the use of a new type of trawel at mid-depths. This net, held open by three "boards," is triangular at the mouth. It spreads fifty feet on a side at the mouth, and trails some two hundred feet behind the boards. It is by far the biggest net which has been tried out at depths below a thousand fathoms.

Other work completed by the "Atlantis" during

(Continued on Page 38)

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Martin Bronfenbrenner, Elizabeth Jenkins, Margaret Mast and Annaleida S. van't Hoff Cattell.

Printed by the Darwin Press, New Bedford

THE WOODS HOLE LOG

The present number of THE COLLECTING NET will be the last one to contain *The Woods Hole Log* during the present summer. Circumstances make it possible to renew our venture initiated in 1929 of issuing the local news in a separate publication. The first number of this off-shoot will appear next Wednesday or Thursday and it will be published weekly thereafter until the end of August. *The Woods Hole Log* will be edited by Miss Rita Guttman and Mr. Martin Bronfenbrenner, published by THE COLLECTING NET and printed by the Darwin Press in New Bedford. The business affairs of this Woods Hole enterprise will be in charge of Miss Margaret Mast.

Removing the "Log Material" and the local advertisements will leave more space for research reports, lectures and other material of more especial interest to the biologist. In addition we hope soon to be able to further increase the number of pages in THE COLLECTING NET so that we can devote still more space to scientific research.

A few readers of THE COLLECTING NET have thought it unwise to include the local news; others have enjoyed reading it. The former will now be able to escape it; the latter can now have more!

Introducing

DR. AUGUST KROGH, professor of zoophysiology at the University of Copenhagen is "visiting scientist" at the Woods Hole Oceanographic Institution this summer. In 1920 Professor Krogh was awarded the Nobel Prize in Physiology and Medicine for his outstanding work on the physiology of capillaries. He is the author of several books in physiology: "The Respiratory Exchange of Animals and Man," one of the volumes in the English series of Monographs on Biochemistry; a text-book for college students in human physiology which has been recently translated into the English language by Dr. Katherine R. Drinker; "The Physiology of the Capilaries." The latter volume is based upon a series of lectures which Dr. Krogh delivered under the auspices of the Silliman Foundation at Yale University in 1922.

Dr. Krogh came over to this country as one of the distinguished foreign guests of the American Association for the Advancement of Science which has recently completed its meetings on the grounds of the Century of Progress Exposition at Chicago. He drove from New York to these meetings with Dr. Walter Mills who is professor of psychology at Yale University.

Dr. and Mrs. Krogh will continue their studies at the Woods Hole Oceanographic Institution through most of August on the organic substances present in sea water and their possible rôle in the nutrition of animals. They plan to sail on August 24 on one of the boats of the Swedish American Line. Sometime during the summer Dr. Krogh will deliver an evening lecture, and his wife will present the results of their newer work in a seminar report.

Mrs. Krogh is examiner in physiology at the University of Copenhagen and also lecturer in physiology and nutrition at the State Normal School for Teachers in Copenhagen. She is interested especially in medical physiology, and her primary problem is the study of the relation between the thyroid and pituitary gland in the guinea pig.

THE "ATLANTIS" AND ITS OCEANOGRAPHIC WORK

(Continued from Page 37)

the past winter can be summarized as follows:

1. Five coastal sections between Cape Hatteras and Cape Cod.
2. A section from the mouth of Chesepeake Bay to Bermuda.
3. A section from Bermuda to Nassau in the Bahamas.
4. Four sections extending from the coast out across the Gulf stream between the Straits of Florida and Cape Hatteras.

It can be seen that the ship has been kept busy and that she is fast completing a general chemical and hydrographic survey of the western half of the North Atlantic.

M. B. L. Calendar

(Continued from 1st page)

TUESDAY, JULY 11, 8:00 P. M.

Lecture: Professor Svedberg, University of Upsala: "Ultracentrifugal and cataphoretic studies on respiratory proteins."

FRIDAY, JULY 14, 8:00 P. M.

Seminar: Dr. M. M. Brooks: The effect of respiratory poisons and methylene blue on cleavage of certain eggs.

Dr. Laurence Irving: Ionic changes during the development of fish eggs.

Dr. E. Newton Harvey: The tension at the surface of egg cells.

Dr. Robert Chambers: A peculiar feature of the cleavage furrow in Arbacia eggs shown in a motion picture film.

DIRECTORY FOR 1933

KEY

Laboratories		Residence	
Botany Building	Bot	Apartment	A
Brick Building	Br	Dormitory	D
Lecture Hall	L	Drew House	Dr
Main Room in Fisheries Laboratory	M	Fisheries Residence	F
Old Main Building	OM	Homestead	Ho
Rockefeller Bldg.	Rock	Hubbard	H
		Kahler	Ka
		Kidder	K
		Whitman	W

In the case of those individuals not living on laboratory property, the name of the landlord and the street are given. In the case of individuals living outside of Woods Hole, the place of residence is given in parentheses.

MARINE BIOLOGICAL LABORATORY

INVESTIGATORS

Abramowitz, A. A. fel. biol. Harvard. Br 108. Ka 23.
Adams, E. M. grad. biol. Cincinnati. Br. 8 Dr 2.
Amberson, W. R. prof. phys. Tennessee. Br 109. Quisset.
Anderson, R. S. res. assoc. phys. Princeton. Br 110. McInnis, Millfield.
Armstrong, Louise S. res. asst. anat. Cornell Med. L 22. D 315.
Armstrong, P. B. asst. prof. anat. Cornell Med. Br 318. D 315.
Atlas, M. asst. zool. Columbia. Br 314. Dr 5.
Baden, V. univ. scholar zool. Pennsylvania. Rock 6. D 210.
Bailey, R. J. instr. zool. George Wasington. OM Base. Stewart, School.
Baitsell, G. A. prof. biol. Yale. Br 323. Brooks.
Ball, E. G. assoc. physical chem. Hopkins Med. Br 110. D 309.
Barron, E. S. G. asst. prof. biochem. Chicago. Br. 207. D 207.
Barth, L. G. instr. emb. Columbia. Br 111. Hubbard, East.
Beck, L. V. grad. asst. phys. Pittsburgh. Rock 7. Dr.
Beckwith, Cora J. prof. zool. Vassar. A 209.
Bell, H. P. prof. bot. Dalhousie. (Canada) Bot 23. D 216.
Bissonnette, T. H. prof. biol. Trinity (Connecticut) OM 26. D 211.
Bostian, C. H. asst. prof. zool. North Carolin State. Rock 3. Dr 5.
Bowen, R. E. asst. prof. biol. Long Island. Br 126. Howes, Water.
Bowling, Rachel instr. proto. Columbia. OM 21. A 307.
Boyden, Louise E. ed. sec. "Biol. Bul." Br 305. A 306.
Bridges, J. C. instr. zool. Morehouse. L 33. A 106.
Brinley, F. J. asst. prof. zool. North Dakota State. OM 38. D 201A.
Brooks, Matilda M. res. assoc. biol. California. Br 233. Gosnold.
Brooks, S. C. prof. zool. California. Br 233. Gosnold.
Brown, W. R. grad. biochem. Cincinnati. Br 342. D 109.
Budington, R. A. prof. zool. Oberlin. Br 218. Orchard.
Butler, E. G. assoc. prof. biol. Princeton. Br 303. D 201.
Butt, C. tech. Princeton. Br 116. Dr.
Calkins, G. N. prof. proto. Columbia. Br 331. Buzzards Bay.
Cannan, R. K. prof. chem. N. Y. Univ. and Bellevue Hosp. Med. Br 310. Gardiner.
Carleton, B. H. asst. phys. Rochester. Br 340. Dr.
Cattell, W. assoc. ed. "Scientific Mo." Br 344. North.
Chambers, R. res. prof. biol. New York. Br 328. Gosnold.
Chao, I. grad. phys. Chicago. Br 315. D 106.
Cheney, R. H. prof. biol. Long Island. Br 126. D 308.
Chesley, L. C. asst. biophysics. Memorial Hosp. Br 343.
Chidester, F. E. prof. zool. West Virginia. Br 344. D 318.
Chute, A. L. Toronto Med. (Canada) OM. 9. Ka. 3.
Clark Eleanor L. vol. invest. Pennsylvania Med. Br 117. West.
Clark, E. R. prof. anat. Pennsylvania. Br 117. West.
Clark, Frances Sec. to dir. Lilly Res. Labs. Br. 328 B. Howe, Main.
Clark, Jean M. grad. Pennsylvania. Br 219. H 4.
Clowes, G. H. A. dir. Lilly Res. Labs. Br 328 B. Shore.
Coe, W. R. prof. biol. Yale. Br 323. A 201.
Coghill, G. E. mem. Wistar Inst. Anat. and Biol. Br 220. Veeder, West.
Coghill, Muriel grad. zool. Denison (Ohio) Br 220. Veeder, West.
Cohen, Rose C. grad. asst. zool. Cincinnati. L 29. H 6.
Conklin, E. G. prof. biol. Princeton. Br 321. High.
Corson, S. A. grad. res. asst. phys. Pennsylvania Med. Br 205. Young, West.
Costello, D. P. instr. zool. Pennsylvania. Br 217n. Elliot, Center.
Crampton, C. B. instr. biol. Wesleyan. Br 210. D 110.
Croasdale Hannah T. grad. bot. Pennsylvania. Bot. 22. W G.
Dan, K. grad. zool. Pennsylvania. Br 111. Clark, Millfield.
Day, Dorothy asst. prof. bot. Smith. Bot. A 205.
Denny, Martha grad. Radcliffe. Br 312. Kittila, Bar Neck.
Donaldson, G. C. prof. anat. Pittsburgh Med. Br 115. Crow Hill.
Donaldson, H. H. Wistar Inst. Br 115. Belfry, Buzzards Bay.
Doyle, W. L. res. asst. phys. Hopkins. Br. 332. Dr 6.
Duncan, P. M. grad. zool. Pennsylvania. Rock 6. K 9.
Drumtra, Elizabeth asst. zool. Barnard. Br 314. K 3.
Eastlick, H. L. asst. zool. Washington (St. Louis) Br 217g. Grave, High.
Edwards, D. J. assoc. prof. phys. Cornell. Med. Br. 214. Gosnold.
Edwards, D. V. assoc. prof. phys. Cornell Med. Br 214. Gosnold.
Edwards, T. I. instr. biol. Hopkins. L 21. Dr 7.
Engel, F. L. Dartmouth. Br 109. D 111.
Engel, G. L. Dartmouth. Br 309. D 111.
Farrow, J. G. grad. zool. Pennsylvania. Rock. Purdam, Main.

Finley, H. E. assoc. prof. biol. West Virginia State L 30. A 105.
Fisher, K. C. fel. Toronto (Canada). Br. 107. Ka 1.
Fleisher, M. S. prof. bact. St. Louis Med. Br 304. D 112.
Fowler, Virginia M. asst. bot. Barnard. Bot. K 3.
Francis, Dorothy S. res. asst. biophysics. Memorial Hosp. Br 343. W F.
Fry, H. J. prof. biol. New York. OM Base. Purdum, Woods Hole.
Furtos, Norma C. fel. biol. Western Reserve. OM Base k. H 1.
Garrey, W. E. prof. phys. Vanderbilt Med. Br 215. Gardiner.
Gerard, R. W. assoc. prof. phys. Chicago. Om 3. D 303.
Gilmore, Kathryn A. instr. bot. Pennsylvania Col. for Women. Rock 3. K 2.
Goldforb, A. J. prof. biol. City N. Y. Br 122C. A 302.
Goodrich, H. B. prof. biol. Wesleyan. Br 210. D 110.
Grand, C. G. res. asst. biol. New York. Br 328. McLeish.
Grave, B. H. prof. zool. DePauw. Br 234. High.
Grave, C. prof. zool. Washington (St. Louis). Br 327. High.
Harnly, Marie L. asst. biol. New York. Br 1. A 102.
Harnly, M. H. asst. prof. biol. New York. Br 1. A 102.
Harvey, Ethel B. independ. invest. phys. Princeton. Br 116. Gosnold.
Harvey, E. N. Osborn prof. biol. Princeton. Br 116. Gosnold.
Harwood, E. M. res. asst. Blue Hill Meteorol. Obs. 315. Young, Middle.
Hayes, F. R. assoc. prof. zool. Dalhousie (Canada). OM 45. D 213.
Hegnauer, A. H. asst. phys. Rochester Med. Br 340. Dr.
Heilbrunn, L. V. assoc. prof. gen. phys. Pennsylvania. Br 219. Spaeth, Whitman.
Henshaw, P. S. biophysics. Memorial Hosp. Br 3. D 206.
Hetherington, W. A. fel. zool. Hopkins. Br 329A. Gray, High.
Hotchkiss, Margaret instr. bact. N. Y. Homeopathic Med. 201. Wilde, Gardiner.
Hibbard, Hope assoc. prof. zool. Oberlin. Br 218. K 12.
Hicks, F. J. grad. zool. Pittsburgh. Rock 7. Ka 21.
Hill, E. S. res. asst. physical chem. Rockefeller Inst. Br 207. D 218.
Hill, S. E. asst. phys. Rockefeller Inst. Br 209A. Veeder, West.
Hoadley, L. prof. zool. Harvard. Br 312. D 315b.
Hoijer, Dorothy J. Chicago Br 207. Neal, West.
Hollaender, A. Nat. Res. fel. biol. Wisconsin. Br 225. Sylvia, Buzzards Bay.
Hoppe, Ella N. res. biologist. N. Y. State Dept. Health. Br 122B. D 313.
Howe, H. E. ed. "Ind. and Eng. Chem." Br 203. Tinkham, West.
Hunter, Laura N. grad. zool. Pennsylvania. Rock 6. Broderick, North.
Hussey, Kathleen L. asst. zool. Connecticut OM Base. W C.
Hutner, S. H. grad. Cornell. OM Base. K 5.
Hyde, Ida H. emer. prof. phys. Kansas State. L 34. Nickerson, Millfield.
Irving, L. assoc. prof. phys. Toronto (Canada). Br. 107. A 202.
Jacobs, M. H. prof. gen. phys. Pennsylvania. Br 102. Sippewissett.
Jao, C. C. grad. bot. Michigan. Bot 26. Dr 9.
Jenkins, G. B. prof. anat. George Washington. OM 34. Clapp, Gardiner.
Johlin, J. M. assoc. prof. biochem. Vanderbilt Med. Br 206. Park.
Johnson, Arline C. grad. asst. zool. Oberlin. OM Base g. H C.
Jones, N. instr. sci. drawing. Swarthmore. Br 211. Hall, Main.
Kaliss, N. Columbia. Br 314. McLeish, Millfield.
Kekwick, R. A. London fel. phys. Princeton. Br 127. Minot.
Kelly, T. L. prof. education. Harvard. OM 120. D 310.
Keltch, Anna K. res. chem. Lilly Res. Labs. Br 319. McInnes, Millfield.
Keil, Elsa instr. zool. N. J. Col. for Women. Br 8. W. D.
Kidder, G. W. tutor biol. City N. Y. Br 217. D 307.
Kirkpatrick, T. B. assoc. prof. physical education. Columbia. L 27. Nickerson, Millfield.
Korr, I. M. asst. instr. biol. Princeton. Br 110. Young, West.
Krogh, Marie lect. phys. & nutrition. State Sch. for Teachers (Copenhagen). 105. D 301.
Kyle, J. A. Br 107. D 108.
Lancefield, D. E. assoc. prof. zool. Columbia. Br 333. Danchakoff, Gardiner.
Lancefield, Rebecca C. assoc. bact. Rockefeller Inst. Br. 208. Danchakoff, Gardiner.
Landowne, M. Harvard Med. Br. 108. Ka 24.
Liedke, Kathe B. grad. zool. Columbia. Br 314. Sylvia, Buzzards Bay.
Lillie, F. R. prof. zool. Chicago. Br 101. Gardiner.
Lillie, R. S. prof. gen. phys. Chicago. Br 326. Gardiner.
Lucke, B. prof. path. Pennsylvania. Br 311. Minot.
Lynch, Ruth S. instr. zool. Hopkins. Br 336. A 101.
MacDougall, Mary S. prof. zool. Agnes Scott. A 208. L 28.
McLane, Kathryn E. instr. biol. Annapolis High School. phys. H 7.
Magruder, S. A. grad asst. zool. Cincinnati. L 29. Kittila, Bar Neck.
Manery, Jeanne F. res. asst. phys. Toronto (Canada). Br 107. H 2.
Marsland, D. A. asst. prof. biol. New York. Br 339. A 102.
Martin, E. A. chairman dept. biol. Brooklyn. OM 39. Newman, Prospect.
Mast, S. O. prof. zool. Hopkins. Br 332. Minot.
Mathews, A. P. prof. biochem. Cincinnati. Br 342. Buzzards Bay.
Maxwell, Jane. instr. biol. Carnegie Inst. Tech. Rock 3. K 2.
Mazia, D. grad. zool. Pennsylvania. Br 219. Ka 24.
Melampy, R. M. asst. animal nutrition. Cornell. OM Base. Dr 10.
Metzner, J. J. grad. proto. Columbia. Br 331. White, Millfield.
Michaelis, L. mem. Rockefeller Inst. Br 207. D 209.
Miller, F. W. grad asst. zool. Pittsburgh. Rock 3. K 15.
Moment, G. B. instr. biol. Goucher. Stewart, School.
Moreland, F. B. res. asst. biochem. Vanderbilt Med. Br. 206. Dr 5.
Morgan, Lilian V. independ. invest. genetics Calif. Inst. Tech. Br 320. Buzzards Bay.
Morgan, T. H. prof. biol. Calif. Inst. Tech. Br 320. Buzzards Bay.
Morrill, C. V. assoc. prof. anat. Cornell Med. L 24. Cape Codder, (Falmouth).
Morris, S. grad. zool. Pennsylvania. Br 217m. D 305
Nelsen, O. E. instr. zool. Pennsylvania. OM 27. D 306.

Newton, Helen K. ms. ed. "Ind. and Eng. Chem." Br 203. Veeder, Millfield.

Nichol, Margaret A. grad. gen. Pennsylvania Col. for Women. Rock 3. W A.

Nonidez, J. F. asst. prof. anat. Cornell Med. Br 318. Whitman.

Novikoff, A. B. fel. biol. Columbia. Br 314. Dr 1.

Orbison, Agnes M. assoc. prof. biol. Elmira. OM 35. Nickerson, Millfield.

Packard, C. asst. prof. zool. Columbia Inst. Cancer Res. OM 2. North.

Palmer, A. H. instr. Univ. & Bellevue Hosp. Med. Br 310. Truslow, Gardiner.

Palmer, Elizabeth T. instr. chem. Vassar. Br 110 g. Truslow, Gardiner.

Parker, G. H. prof. zool. Harvard. Br 213. A 309.

Parpart, A. K. asst. prof. phys. Princeton. Br 205. Minot.

Pelluet, Dixie asst. prof. zool. Dalhousie (Canada). OM 45. D 103.

Plough, H. H. prof. biol. Amherst. Br 204. Whitman.

Pollister, Priscilla F. instr. Brooklyn. OM 44. D 314.

Porter, Helen. asst. zool. Harvard. Br 213. Grinnell, Bar Neck.

Prescott, G. W. asst. prof. biol. Albion. Bot 25. D 101.

Rex, R. O. instr. anat. Pennsylvania. Br 117. Conklin, High.

Richards, O. W. instr. biol. Yale. Br 8. A 303.

Richardson, Margaret S. Brearley School. Br 318. Hubbard, Center.

Root, W. S. assoc. prof. phys. Syracuse Med. Br 226. Oak, Park.

Rubenstein, B. B. asst. phys. Chicago. Br. 309. D 106.

Rugh, R. instr. zool. Hunter. Br 111. D 208.

Rusch, Elizabeth res. asst. biophysics. Memorial Hosp. Br 343. Densmore, School.

Russell, W. L. grad. genetics. Amherst. Br 204.

Sauer, F. C. asst. prof. zool. Wichita. Br. 217. Thompson, Main.

Schechter, V. instr. invert. zool. City N. Y. OM 1. Dr 2.

Schweitzer, M. D. grad. zool. Br 333. McLeish, Millfield.

Scott, A. C. asst. zool. Columbia. Br. 314. Thompson, Main.

Sell, J. P. grad. asst. biol. Yale. Rock 7. Ka 21.

Shapiro, H. grad. biol. Princeton. Br 127. Edwards, School.

Shaw, I. res. asst. biol. Long Island. Br 126.

Shoup, C. S. asst. prof. biol. Vanderbilt. Br 315. D 301B.

Sichel, F. J. M. asst. instr. biol. New York. Br 339. Dr.

Slifer, Eleanor H. res. assoc. zool. Iowa. Br 217A. Kittila, Bar Neck.

Sonneborn, T. M. res. assoc. zool. Hopkins. Br 336. D 204.

Specht, H. grad. phys. Hopkins. OM Base. Dr 6.

Speicher, B. R. grad. asst. zool. Pittsburgh. Rock 3. K 15.

Speidel, C. C. prof. anat. Virginia. Br 106. D 104.

Spek, J. prof. zool. Heidelberg (Germany). Br 223. D 316.

Stabler, R. M. instr. zool. Pennsylvania. OM 22. D 210.

Starkey, W. F. grad. zool. Pittsburgh. Rock 7. Ka 21.

Stewart, Dorothy R. asst. prof. biol. Skidmore. Br. 222. Stokey, Gardiner.

Stockard, C. R. prof. anat. Cornell Med. Br 317. Buzzards Bay.

Strong, O. S. prof. neur. and neuro-hist. Columbia. Br 8. Elliot, Center.

Stuart, Martha S. grad. genetics. Pennsylvania Col. for Women. Rock 3. W A.

Summers, F. M. tutor biol. City N. Y. Br 2171. A 104.

Sumwalt, Margaret asst. instr. phys. Pennsylvania. OM 3. W G.

Tashiro, S. prof. biochem. Cincinnati. Br 341. Park.

Taylor, G. W. Nat. Res. fel. phys. Princeton, Br 110. Cowey, School.

Taylor, W. R. prof. bot. Michigan. Bot 24. Whitman.

Wade, Lucille W. grad. biol. Hopkins Sch. Hygiene. Br 319. W. I.

Walker, P. A. fel. Harvard. Br 312. Thompson, Water.

Wallace, Edith M. scientific artist. Calif. Inst. Tech. Br 320. Main.

Waterman, A. J. instr. biol. Brooklyn. OM 39. D.

Weisman, M. N. tutor biol. City N. Y. Br 217J. McLeish, Millfield.

Whiting, Anna R. prof. biol. Pennsylvania Col. for Women. Rock 3. Whitman.

Whiting, P. W. prof. zool. Pittsburgh. Rock 3. Whitman.

Wieman, H. L. prof. zool. Cincinnati. Br 334. D 311.

Willey, C. H. asst. prof. biol. New York. Br 232. A 301.

Wilson, E. B. DaCosta prof. emeritus zool. Columbia. Br 322. Buzzards Bay.

Wilson, Hildegard, N. asst. biochem. Univ. & Bellevue Hosp. Med. Br 310. Buzzards Bay.

Winsor, C. P. grad. phys. Harvard. L 21. (Cataumet).

Wolf, E. A. assoc. prof. zool. Pittsburgh. Rock. 7. Elliot, Center.

Woodruff, L. L. prof. proto. Yale. Br 323. Gansett.

Young, Roger A. asst. prof. zool. Howard. Br 315. A 304.

Young, W. C. asst. prof. biol. Brown. OM 34. Kittila, North.

Zirkle, C. assoc. prof. bot. Pennsylvania. Bot 6. A 101.

Zujko, A. J. asst. biol. Trinity (Connecticut) OM 26. Ka 23.

STUDENTS

Albaum, H. G. fel. biol. Brooklyn. emb. Dr 1.

Alt, H. L. assoc. med. Northwestern Med. phys. D 217.

Amidon, Elaine W. Syracuse. bot. H8.

Armack, C. M. curator biol. Mus. Northern Arizona. emb. Crowell, Water.

Bates, M. N. grad. asst. zool. Oberlin. emb. Dr 2..

Bechtel, W. R. instr. biol. Edinburg High Sch. (Ohio) proto. Bosworth, North.

Bell, Ruth grad. asst. zool. Wellesley. emb. W B.

Bengel, W. Z. asst. anat. & emb. DePauw. emb. Ka 2.

Bosworth, M. W. asst. biol. Wesleyan. bot. K 6.

Botsford, E. Frances asst. prof. zool. Connecticut. phys. Stokey, Gardiner.

Boyer, D. C. grad. biol. Columbia. proto. Hilton, Millfield.

Campbell, Mildred F. teach. bot. Shortridge High Sch. (Indianapolis) bot. Hall, Main.

Chen, Y. grad. zool. Pennsylvania. emb. Elliot, Center.

Churney, L. grad. zool. Pennsylvania. emb. Ka 2.
Cunniff, Hilda S. bot. H 9.
Dennis, Nova N. teach. biol. Portage County High Sch. (Ohio) proto. Clark, Millfield.
Derrickson, Mary B. phys. W D.
DeWolf, R. A. instr. zool. Rhode Island State. emb. (Hyannis.)
Foster, Edith F. Vassar. emb. Bosworth, North.
Glassmeyer, E. J. grad. biochem. Cincinnati. phys. D 109.
Godwin, M. C. asst. hist. & emb. Cornell. emb. K 7.
Greco, F. M. Hunter. emb. Kittila, Bar Neck.
Hamilton, Mary A. Elmira. emb. H 7.
Havey, C. B. Acadia (Canada) phys. Dr 1.
Hibbard, Jeanne Oberlin. phys. K 12.
Hirschfield, N. B. Univ. and Bellevue Hosp. Med. proto. McLeish, Millfield.
Hooper, Kathryn T. Wheaton. emb. Young, West.
Howell, C. D. grad. biol. Hopkins. phys. Dr.
Johnson, Edna L. assoc. prof. biol. Colorado. phys. A 305.
Kagan, B. M. Washington & Jefferson. emb. K 6.
Kriete, F. M. DePauw. emb. K 9.
Lippman, R. W. Yale. emb. Hilton, Main.
McAuley, A. A. DePauw. emb. K 9.
McGehee, Elise grad. Newcomb. emb. Oak, Park.
MacIntosh, F. C. dem. pharm. Dalhousie (Canada) phys. Thompson, Water.
Mathews, R. S. Physicians & Surg., Columbia. phys. Dr 6.
Melampy, R. M. asst. anim. nutrition Cornell. phys. Dr 10.
Moreland, F. B. grad. res. asst. biochem. Vanderbilt Med. Phys. Dr 5.
Moser, F. grad. zool. Pennsylvania. emb. D 214.
Perkins, Irene T. grad. biol. Columbia. proto. A 204.
Poris, Ethel Hunter. bot. Kittila, Bar Neck.
Ramey, Sally Elmira. bot. Bosworth, North.
Root, Charlotte M. Ohio Wesleyan. emb. H 6.
Rose, S. M. grad. asst. biol. Amherst. emb. K.
Ross, E. grad. physico-chem. biol. California. phys. Ka 2.
Rubidge, Karyl W. Vassar. emb. Bosworth, North.
Solandt, D. Y. res. fel. phys. Toronto (Canada) phys. D 107.
Solandt, O. M. res. asst. phys. Toronto (Canada) phys. D 107.
Spangler, Betty A. Wheaton. emb. Young, West.
Stricker, G. J. Yale. phys. Neal, West.
Stubbs, T. H. instr. biol. & chem. Emory. proto. Sylvia, Buzzards Bay.
Summers, F. M. grad. proto. Columbia. Br 217. A 104.
Sweadner, W. R. grad. asst. Pittsburgh. Rock 3. Dr 8.
Taylor, H. C. grad. asst. biol. Wesleyan. emb. K.
Tukey, Gertrude R. Smith. emb. H 7.
Turner, R. S. instr. biol. Dartmouth. emb. K.
Urban, J. instr. biol. Randolph High Sch. (Ohio) proto. Bosworth, North.
Vexler, D. E. grad. phys. Rutgers. phys. Ka 22.
Ward, Mary Wellesley. proto. Grinnell, West.
Wardwell, Judith S. grad. asst. zool. Wellesley. emb. W B.
Webster, M. Dorothy grad. bot. Dalhousie. (Canada) phys. Young, West.
Young, M. I. instr. biol. Junior Col. Augusta. proto Sylvia, Buzzards Bay.
Zinn, D. J. Harvard. emb. D 108.

ADMINISTRATION OFFICE

Billings, Edith secretary. Millfield.
Crowell, Polly L. asst. to bus. mgr. Main.
Dillinger, Bessie R. secretary. Br 104 b. K 8.
Karr, Dorothea secretary. W E.
MacNaught, F. M. bus. mgr. School.

LIBRARY.

Endrejat, Doris assistant. W H.
Lawrence, Deborah secretary. Locust (Falmouth).
Montgomery, Pricilla B. librarian. Whitman.
Rohan, Mary A. assistant. Millfield.

SPECIAL APPARATUS AND TECHNICAL SERVICE

Adams, E. M. grad. biol. Cincinnati. Chem. D 2.
Apgar, A. R. Br 211. photographer. D 105.
Boss, L. F. res. tech. Middle.
Callahan, J. Janitor. Ka. 3.
Chute, A. L. Toronto Med. (Canada) asst. Ka. 3.
Cornish, G. janitor. Br 1st floor. Dr 4.
Densmore, S. gardener. School.
Frew, Pauline. Bates. Chem. W. F.
Googins, H. janitor. Quisset.
Graham, J. D. glass-blowing. Veeder, Millfield.
Hemenway, W. carpenter. Carpenter Shop. Hawthorne.
Johlin, Sally Wellesley. Chem. Gardiner.
Kahler, R. MBL asst. Br 7. Glendon.
Keil, Elsa instr. zool. N. J. Col. Women. Chem. W D.
Keltch, R. janitor. Br 3rd floor. Millfield.
Larkin, T. superintendent. Br 7. Woods Hole.
Laug, E. P. instr. phys. Pennsylvania. Chem. D 302.
Liljestrand, P. H. Ohio Wesleyan. asst. Dr 3.
Liljestrand, R. S. Cazenovia Sem. night watch. Ka 4.
McInnis, F. M. janitor. Bot & L. Millfield.
McManus, J. janitor. Br 2nd floor. Ka 3.
Mast, Louise grad. biol. Hopkins. Minot.
Meier, O. Pennsylvania. night eng. Dr 15.
Pond, S. E. asst. prof. phys. Pennsylvania. tech. mgr. Queens (Falmouth).
Richards, O. W. instr. biol. Yale. Chem. In charge. A 303.
Sander, M. Pennsylvania. res. tech. Dr 14.
Strong, O. S. prof. neur. & neuro-hist. Chemist emeritus. Elliot. Center.
Swain, G. R. janitor. Br 3rd floor. Main (Quisset).
Tawell, T. E. head janitor. Br Base. Thompson, Water.
Tupper, Mary C. Swarthmore. Chem. W H.

SUPPLY DEPARTMENT

Clarkson, W. collector. Water.
Crowell, Ruth S. secretary. Main.
Crowell, P. S. Harvard. collector. School.
Erlanger, H. Wisconsin. collector. Dr 3.
Gray, G. M. Curator res. mus. Buzzards Bay.
Gray, M. collector. (Teaticket).
Hanau, R. collector. Dr 3.
Hilton, A. M. collector. Millfield.
Leathers, A. W. head shipper. Minot.
Lehy, J. collector. Millfield.
McInnis, J. resident mgr. Millfield.
Neilsen, Anna M. secretary. Millfield.
Pratt, M. collector. Dr 3.
Sither, J. A. Wabash. collector. Dr.
Smith, C. B. Syracuse. collector. Supply Dept.
Thornley, W. Harvard. collector. Supply Dept.
Wamsley, F. W. supervisor schools, Charleston. spec. preparator. Supply Dept.

WOODS HOLE OCEANOGRAPHIC INSTITUTION

INVESTIGATORS

Beach, E. F. Brown. 109. Hilton, Water.

Bigelow, H. B. prof. zool. Curator Oceanography. Harvard. 114. Luscombe, Main.

Braarud, T. assoc. phytoplanktonol. Internat. Passamaquoddy Fish. Comm. 311. F.

Brill, E. R. Harvard. 109. Neal, West.

Bruce, W. F. Rockefeller Inst. 201. "Asterias."

Buck, W. B. Princeton. "Asterias."

Carey, Cornelia L. asst. prof. bot. Barnard. 202. Quisset.

Church, P. E. res. asst. 315. Wilde, Gardiner.

Clarke, G. L. instr. gen. phys. Harvard. 108. Mitchell, Orchard.

Emmons, G. Mass. Inst. Tech. 208.

Fish, C. J. exec. sec. Internat. Passamaquoddy Fish. Comm. 310. Buzzards Bay.

Green, Arda A. res. fel. Fatigue Lab. Harvard. 101. D 202.

Greenwood, T. S. tech. W. H. O. I. "Atlantis."

Hotchkiss, Margaret instr. bact. N. Y. Hom. Med. Col. & Flower Hosp. Wilde, Gardiner.

Ingalls, Elizabeth N. res. asst. Harvard. 103. Young, West.

Iselin, C. O. asst. curator oceanography. Harvard. 206. (Falmouth).

Keys, A. A. Nat. Res. fel. phys. Cambridge (England) 106.

Krogh, A. prof. zoophys. Lab. Zoophys. Copenhagen (Denmark). 105. D 301.

Leavitt, B. B. 301*.

Lichtblau, S. 209. Young, West.

Mahncka, H. grad. Brown. 109.

Oster, R. H. Harvard. 106.

Rakestraw, N. W. assoc. prof. chem. Brown. 109.

Redfield, A. C. prof. phys. Harvard. 103. Millfield.

Redfield, J. H. 107. Breakwater. Bar Neck.

Renn, C. E. asst. microbiol. Rutgers. 201. Young, Middle.

Reuszer, H. W. instr. soil microbiol. Rutgers. 201. Young, Middle.

Root, R. W. instr. biol. City N. Y. 101. Cowey, School.

Rossby, C. G. meteorolgist. Mass. Inst. Tech. 207.

Schalk, M. grad geol. Harvard. 211. Stewart, School.

Sears, Mary. 301. Hilton, Water.

Smith. grad. physical chem. Brown. 109.

Stetson, H. C. asst. curator palaeon. Harvard. 212. (Falmouth.)

Taylor, Carola W. res. asst. geol. Radcliffe. 212., Cowey, School.

Turner, A. "Atlantis."

Waksman, S. A. prof. soil microbiol. Rutgers. 202. (Penzance.)

Watson, E. E. hydrographer, Internat. Passamaquoddy Fish. Comm. 310. Lakeview.

Wheeler, C. Harvard. 108. Hatchfield.

Wilson, C. B. 111. Clough, Millfield.

Woodcock, A. tech. W. H. O. I. 207. Peck, Woods Hole.

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*Further information refused. Enquire at Room 301.

OFFICE

Schroeder, W. C. business manager. 113. W.H.O.I.

Walker, Virginia B. secy; asst. bus. mgr. 112. Howe, Millfield.

BUILDINGS AND GROUNDS

Condon, J. W. janitor. Millfield.

Eldridge, S. N. carpenter. Woods Hole.

Schroeder, W. superintendent. W. H. O. I.

Sylvia, A. F. gardener. Millfield.

"ATLANTIS"

Bachus, H. "Atlantis".
Carlson, "Atlantis".
Cavalone, cook "Atlantis".
Condon, J. G. "Atlantis".
Costa, W. mess boy "Atlantis".
Dwyer, E. mess boy "Atlantis"
Kehoe, T. capt. "Asterias".
Kelly, T. 1st mate "Atlantis".
Lindstrom, J. "Atlantis".
McLunin, "Atlantis".
McMurray, F. capt. "Atlantis".
Olsen, B. "Atlantis".
Potter, D. 2nd mate "Atlantis".
Turner, D. "Atlantis".

U. S. BUREAU OF FISHERIES

Bigelow, R. P. prof. zool. Mass. Inst. Tech. Center.

Galtsoff, P. S. biol. U. S. B. F. (Washington) 122. F.

Goffin, R. collector. U. S. B. F. 115. Millfield.

Linton, E. fel. paras. Pennsylvania. M 5 West.

Sette, O. E. director U. S. B. F. (W. H.) 118. F.

Smith, R. O. asst. aquatic biol. U. S. B. F. M 124. F.

SCIENTIFIC STAFF

Bigelow, R. P. prof. zool. Mass. Inst. Tech. Center.

Galtsoff, Eugenia assoc. zool. George Washington 122. F.

Galtsoff, Paul S. biol. U. S. B. F. (Washington) 122. F.

Goffin, R. collector. U. S. B. F. 115. Millfield.

Linton, E. fel. paras. Pennsylvania M5. West.

Sette, O. E. director U. S. B. F. 118. F.

Smith, R. O. asst. aquatic biol. U. S. B. F. F.

BUILDINGS AND GROUNDS

Brown, G. guide. Hatchery.

Conklin, Paul, fireman. Hatchery.

Hoffses, G. R. superintendent 117. F.

Howes, E. S. coxswain. Millfield.

Howes, W. L. fish culturist. Millfield.

Kristtan, M. apprentice fish culturist. Hatchery.

Lowey, J. engineer. Glendon Road.

Radel, A. H. apprentice fish culturist. Hatchery.
Sanderson, A. apprentice fish culturist. Hatchery.
Van Amberg, L. fireman. Hatchery.
Webster, H. fireman. Hatchery.

The A. B. C. of Woods Hole for 1933

All Schedules Set to Daylight Saving Time — Bold Type Indicates P. M.

TRAIN SCHEDULE

WOODS HOLE TO BOSTON

	Daily	Daily	Daily	Sunday	Sunday
Woods Hole	7:15	9:40	**5:40**	**6:10**	**8:10**
Falmouth	7:22	9:47	**5:47**	**6:17**	**8:17**
Boston	9:10	11:52	**7:52**	**8:15**	**10:22**

BOSTON TO WOODS HOLE

	Daily	Daily	Daily	Sat. only	Ex. Sat. and Sun.
Boston	8:15	**1:30**	**4:47**	**1:03**	**4:03**
Falmouth	10:32	**3:35**	**6:48**	**3:00**	**6:02**
Woods Hole	10:40	**3:42**	**6:55**	**3:06**	**6:09**

SEAPLANE SCHEDULE

Eastbound	Trip 1†	Trip 3	Trip 5	Trip 7	Trip 9
Leave New Bedford	7:00	10:30	**12:45**	**3:30**	**5:55**
Leave Woods Hole	flag	10:50	flag	**3:50**	**6:15**
Leave Vineyard Haven	7:30	11:07	**1:15**	**4:07**	**6:30**
Arrive Nantucket	7:50	11:27	**1:35**	**4:27**	**6:50**

Westbound	No. 2†	No. 4	No. 6	No. 8	No. 10*
Leave Nantucket	8:50	11:37	**2:30**	**4:35**	**6:55**
Leave Vineyard Haven	9:20	**12:07**	**3:00**	**5:05**	**7:20**
Leave Woods Hole	9:37	**12:24**	**3:17**	**5:22**	....
Arrive New Bedford	9:47	**12:34**	**3:27**	**5:32**	**7:35**

* Trip 10 runs Tues., Thurs., Sundays only. † Trips 1 and 2 do not run Sundays.

BOAT SCHEDULE

For New Bedford, Woods Hole, Oak Bluffs, Vineyard Haven and Nantucket

Leave	Daily	Daily	Daily	Daily
New Bedford	7:00	9:30	**2:30**	**7:45**
Woods Hole	8:20	10:50	**4:00**	**9:00**
Oak Bluffs	9:10	11:40	**4:45**	....
Vineyard Haven	9:30	12:00	**5:00**	**9:45**
Nantucket	11:30	**2:00**	**7:15**	....

Leave	Daily	Daily	Ex. Sun.	Sun.	Daily
Nantucket	....	6:30	**2:30**	**3:00**	**5:00**
Vineyard Haven	6:10	8:15	**4:00**	**4:30**	**6:55**
Oak Bluffs	....	9:00	**4:30**	**5:00**	**7:15**
Woods Hole	6:55	9:45	**5:20**	**5:50**	**8:00**
New Bedford	8:15	11:15	**6:45**	**7:30**	**9:25**

CONTROL OF THE CAPE COD MOSQUITO

The Cape Cod Mosquito Control Project, sponsored by the Cape Cod Chamber of Commerce and financed largely by public subscription, has been in operation since May, 1930. It is now one of several projects under the State Reclamation Board. The work consisted chiefly of the elimination of salt marsh breeding places by drainage and sometimes by dyking and filling. A relatively small amount of work has been done at fresh water breeding places, due to the fact that the salt marsh species of mosquito is much more numerous. In fact a survey of the mosquito situation made by R. W. Wales (Entomologist) and Percival M. Churchill (Consulting Engineer) for the State Reclamation Board showed that 90 to 95% of all Cape Cod mosquitoes were of species breeding in salt marshes.

In addition to the type of work already mentioned, a truck equipped for spraying oil has been in use since the season of 1931. This truck is used chiefly to combat the house mosquito which breeds in all sorts of places containing water near dwellings, and to oil salt marshes and other breeding places when conditions warrant it.

It is now three years since the project went into operation and the progress made in combating mosquitoes can be measured fairly accurately by comparing the abundance of salt marsh mosquitoes with the fresh water ones. From an estimated 90 to 95% in 1929, the salt marsh mosquitoes have been reduced to a fraction of the number of fresh water mosquitoes. At the present time there are large areas of the Cape where only fresh water species can be found. Formerly the salt marsh species covered every part of the Cape and sometimes cattle, horses and even people would be literally covered with hundreds of mosquitoes. We no longer hear of hotels being closed and people being driven from the golf courses and beaches.

With the salt marsh mosquitoes nearly under control the project will be able to turn its attention more fully to the fresh water species. Unfortunately these mosquitoes often breed in places where control measures are very difficult and expensive. There is also the difficulty caused by the need of flowing water in cranberry bogs in the spring. Mosquitoes hatch in the bogs and in the adjacent flooded areas during the month of May. However, in spite of adverse conditions, if the most available breeding places are eliminated and some of the more important of the others controlled mosquitoes will be very few indeed. At present the house mosquito is one of the worst fresh water species. Its presence is mainly due to the carelessness of the people; in fact the pest is very rarely found away from human habitation. This mosquito breeds in water in barrels, tubs, cans and in all sorts of artificial containers. It is also found in flooded cellars, open cisterns, cesspools, catchbasins and even in harmless pools if garbage or rubbish is thrown in. If people would be careful the Project would need only to oil the catch basins.

The final degree of relief from mosquitoes will depend on how many of the fresh water breeding places can be eliminated, and this bears a direct relation to the amount of money available. At present the funds are rather limited. A small tax collected by the State provides for the up keep and maintenance of the ditching and oiling after the original work is done.

The Commissioners of the Cape Cod Mosquito Control Project are: O. C. Nickerson, of Chatham; F. W. Norris, of Oyster Harbor; L. C. Weeks of Falmouth.

—*Cape Cod Mosquito Control Project.*

Important Meeting of the M. B. L.

The annual meeting of the members of the M. B. L. Club will be held in the Clubhouse on Monday evening, July 10. All members are urgently requested to attend since officers are to be elected and important policies will be decided upon.

A trip to New York in a fourteen-foot outboard motor boat was undertaken last fall by Victor Schechter and his brother. The trip took 4½ days and stops were made at Palmer Island Light, Point Judith, Saybrook Point and Tavern Island. The navigators attended a breeches bouy drill at Point Judith and were escorted across Newport Harbor by a school of porpoises.

The Woods Hole fire station is inspected every fifth day by the Chief and Deputy Chief of the Falmouth Fire Department. The annual General inspection of all equipment and personnel took place on June 1.

A Food Sale was held by the M. E. Woman's League on Friday June 23rd from 3 to 5 P. M. in the M. E. church. The affair was a decided financial, social and gustatorial success.

Mr. Gardner Handy, who formerly drove the bus for Mr. B. K. Nickerson is now working for Penzance Garage.

THE SUPPLY DEPARTMENT OF THE MARINE BIOLOGICAL LABORATORY

By the Staff of the Supply Department

One of the main duties of the Supply Department during the summer season is to give the investigators and students the very best possible service.

The available materials will be collected and delivered to all those who request them. Orders for material to be delivered the following day will be taken between 10:00 A. M. and noon-time. If the investigator who does not expect to be in his room between those hours will leave a notation of what he desires, it will greatly facilitate the service. This may be done by placing a slip on the door; then he may be sure that the boy will take it and the material be delivered.

If there are any complaints about the material or service, it would be greatly appreciated if they were entered in the Supply Department office, instead of being given to the delivery boy or to a member of the crew.

This department is maintained at a very great expense during the summer months. During the winter months, the Supply Department is maintained as a Supply House, where students and teachers may order their needs for their class work. The all-year-round personnel is made up of six collectors, and in the summer this number is increased by eight additional collectors on the crew. Two people are on duty at the office at all times, and they will gladly give any information or adjust any complaints which may be entered.

Few teachers realize the expense that is involved in the collecting and preparing of marine animals. Many, we are certain, believe that it is only necessary to walk along the beach, pick up the specimens and put them into formaldehyde. Nothing could be farther from the truth. The entire collecting region must be carefully explored in order to find sources for the various forms, and at times it is necessary to take long trips to secure them. To do this exploring and collecting, boats costing several thousand dollars must be employed. These must be provided with pumps, so that the specimens may be kept in running sea-water while they are on board. Then, when they are brought to the laboratory, many of them must be put through long and complicated processes to be properly narcotized, expanded and preserved. The pumps and tanks needed to supply the laboratory with running sea-water are very expensive, and far beyond the means of any individual who may be trying to collect without equipment.

The Supply Department has a biology catalogue, which will be given out upon request, and which lists the complete stock of preserved and living material. This may be obtained at the office. The prices of materials have been greatly reduced, and special attention is being called to the grading of the sizes in materials which have been arranged for the convenience of the customers. Should estimates be desired, we will gladly give same, and all orders will have our careful attention.

Our Department is, without doubt, the best equipped marine collecting station in the United States, if not in the world. Its collecting equipment, consisting of boats, fish traps, seines, dredges, tangles and laboratory facilities, are of the very best, and represent a great investment. Its staff of collectors and preparators has had many years of experience. It is these advantages in the collection and preparation of marine specimens which explain, to a great extent, the uniformly good quality of the preserved material furnished by the Supply Department.

RETRENCHMENT AT THE U. S. BUREAU OF FISHERIES

Governmental economy has hit the Bureau of Fisheries hard this year, and has resulted in a decided curtailment of services of the Woods Hole station. It is no longer possible to accommodate the twenty or thirty university investigators who formerly made use of the facilities, and therefore only two investigators, with their assistants, are occupying the laboratories and residence of the local station.

Dr. Paul Galtsoff is continuing his work on oysters and Director Oscar Sette is in charge of the mackerel investigation. Both Fisheries boats, the *Albatross II* and *Phalarope II* are in temporary retirement and are tied up at the wharf. The old *Phalarope*, in the Eel Pond, long a landmark —or should we say seamark—of Woods Hole, has been sold, and has temporarily joined the army of the unemployed. The aquarium and other public exhibits are open only temporarily. Plans for next year are uncertain.

During the winter, the exhibits were enlarged by the gift of the Vinal Edwards collection of stuffed birds from Woods Hole and vicinity. Dr. Edwards was formerly director of the Bureau station at Woods Hole, and his heirs presented the collection to the station last year. It might be noted that all other permanent exhibits in the aquarium building were obtained during Dr. Edwards' long regime as director, either as a result of his personal efforts or during the Biological Survey of the region, carried on independently from 1904 to 1911.

LECTURES AND SYMPOSIA AT COLD SPRING HARBOR

We have received from the Long Island Biological Laboratory the following announcement of the lectures and symposia on "The Potential Difference at Interfaces and Its Bearing Upon Biological Phenomena" at Cold Spring Harbor:

SATURDAY, JULY 1: Hans Muller; The theory of the diffuse double-layer.

MONDAY, JULY 3: Hans Muller; The theory of cataphoretic migration. D. R. Briggs; Streaming potential measurements. Kenneth S. Cole; Surface conductance.

WEDNESDAY, JULY 5: SYMPOSIUM; OXIDATION-REDUCTION POTENTIALS. D. A. MacInnes; Meaning and calibration of the pH scale. L. Michaelis; The reversible two-step oxidation. Barnett Cohen; Reversible oxidation-reduction potentials in dye systems and their use in the examination of cells and cell suspension. I.

WEDNESDAY, JULY 12: SYMPOSIUM; BIOELECTRICAL PHENOMENA. Hans Muller; The theory of ionic adsorption. W. J. V. Osterhout; Bioelectrical phenomena in large cells. Kenneth S. Cole; Electric excitation in nerve. Herbert S. Gasser; Axon action potentials in nerve.

WEDNESDAY, JULY 19: SYMPOSIUM; ELECTRICAL PROPERTIES OF SURFACES IN RELATION TO THE COAGULATION PROCESS. Hans Muller; Stability of colloids and the theory of rapid coagulation. Harold Abramson; The chemical constitution of amphoteric surfaces. (Amino-acids; proteins). Stuart Mudd; Agglutination.

MONDAY, JULY 24: SYMPOSIUM; OSMOTIC PHENOMENA. D. R. Briggs; Electrosmosis and anomolous osmotic pressures. W. J. V. Osterhout; Diffusion and osmosis in cells and models. Eric Ponder; Osmotic behavior of red cells. I. Robert Chambers; Intracellular oxidation-reduction potentials.

WEDNESDAY, JULY 26: SYMPOSIUM; ELECTRICAL PROPERTIES OF THE RED CORPUSCLE. Hugo Fricke; Electric capacitance and conductance of red blood cells with an application to the study of hemolysis. Harold Abramson; The electrical charge of the blood cells of the horse and its relation to the inflammatory process. D. D. Van Slyke; Factors controlling the electrolyte and water distribution in the blood. Eric Ponder; Osmotic behavior of red cells. II.

FRIDAY, JULY 28: Kenneth S. Cole; Electrical conductance of biological material. Barnett Cohen; Reversible oxidation-reduction potentials in dye systems and their use in the examination of cells and cell suspension. II. Stuart Mudd; Phagocytosis.

NOTES ON THE WORK OF THE M. B. L. CLUB

Nearly two hundred people attended the "mixer" and dance last Saturday evening. A four-piece orchestra began playing at ten-thirty, and the floor was over-crowded with couples until much later in the evening when some of them went home. The refreshment part of the program was in charge of Miss Margaret Mast who had punch served to the thirsty group.

Any worker at the three scientific institutions at Woods Hole is cordially invited to attend the Saturday-night dances. No charge will be made to them providing they are members of the Club. They may bring guests who are not connected with the laboratories, but guests of members will be classified in the "non-member" group and be subject to a charge of fifty cents. Thus anyone who has not contributed directly toward the support of the Club will be expected to share in the expense of providing an orchestra.

The weekly victrola-record concerts are expected to begin next Wednesday. Notices suggesting that people loan records have been posted at the Laboratory and at various other points in the village.

In order to eliminate the destruction of club property and the missuse of its facilities (which has been brought about almost entirely by non-members) Mrs. Morris has organized a number of investigators to serve as "host and hostess" during the evenings in July and August. Those which she has appointed for the coming week are:

Monday, July 10—Mr. and Mrs. Ware Cattell.

Tuesday, July 11—Dr. and Mrs. Edwin Linton.

Wednesday, July 12—Mr. and Mrs. Samuel Morris.

Thursday, July 13—Dr. and Mrs. Möyer S. Fleisher.

Friday, July 14—Dr. and Mrs. G. B. Jenkins.

Saturday, July 15—Dr. and Mrs. R. M. Stabler.

Sunday, July 16—Dr. and Mrs. Samuel Schoop.

The primary duty of these individuals will be to make people feel at home in the Club, and to help the members meet each other.

The next expansion of the activities of the M. B. L. Club is to be the acquistion of a ping-pong table. It will be the source of much amusement, especially during evenings when the weather is poor. A ping-pong tournament is scheduled for later in the summer.

ITEMS OF INTEREST

FREDERICK L. GATES

The sudden death of Dr. Frederick L. Gates, a worker at the Marine Biological Laboratory, shocked Woods Hole residents in June, especially those associated with him and his scientific work. A fall in his laboratory at Harvard University resulted in a skull fracture, and Dr. Gates died in Boston on June 17. He was forty-six years of age.

Dr. Gates was born in Minneapolis in 1886, and after completing a college course at Yale and taking a medical degree at Johns Hopkins, he embarked upon a career of biological research at the Rockefeller Institute, which his father had helped induce Mr. Rockefeller to endow. He remained at the Institute for seventeen years, becoming an associate member before he accepted the lectureship in physiology at Harvard three years ago.

At the time of his death Dr. Gates was investigating the effects of ultra-violet light of different wave lengths on living tissues of various kinds. He worked at the Rockefeller Institute with Dr. Peter Olitsky on several types of bacteria, with a view to discovering the causes of common colds and influenza.

Dr. Gates' home here was on Nobska Road. His family left at the end of June for Minnesota where they will spend the summer.

Dr. Caswell Grave has left Woods Hole for six weeks in the Tortugas. Mr. Paul Nicoll is accompanying him as an assistant. The tunicates of the region will be the objects of their study.

Dr. and Mrs. W. J. V. Osterhout (the former Miss Marian Irwin) have taken a house at Halesite, Huntington, Long Island, for the summer.

Dr. H. B. Bigelow, director of the Woods Hole Oceanographic Institution, attended the International Council for the Exploration of the Sea in Paris from May 8-13. He is now at Woods Hole.

Dr. Henry Knower has been appointed Research Associate in Biology at the Osborn Zoological Laboratory at Yale.

Miss Lois E. Te Winkel has been appointed instructor at Smith College for the coming academic year. She will give a course in embryology and will assist in the mammalian anatomy course.

THE COLLECTING NET SCHOLARSHIPS

Dr. H. B. Goodrich, professor of biology at Wesleyan University and director of the course in embryology at the Marine Biological Laboratory, recently made the following statement concerning the value of THE COLLECTING NET Scholarships:

"In my opinion the COLLECTING NET scholarships perform an exceedingly useful service. The students assembled at Woods Hole are carefully selected and are of unusual ability. Candidates from this group are certain to merit aid. The period of graduate study is often the most difficult to finance in the student's career. A scholarship of $100.00 will permit a careful student to pay board or room for ten weeks, or it may in part be applied to laboratory or tuition fees. It is undesirable for many to defray expenses by waiting on table at the 'mess' as this often demands four to five hours a day—or more than can be spared from their working time and energy. The purpose of the scholarships seems to me to be in every way admirable."

(Signed) H. B. GOODRICH.

Miss Elizabeth T. Kinney and Dr. Leonard Worley were married at South Hadley, Mass., on June 17th. Dr. and Mrs. Worley are motoring to Nebraska for their wedding trip.

A daughter was born to Dr. O. E. Sette, director of the laboratory, U. S. Bureau of Fisheries, and Mrs. Sette during the month of March.

New visitors and old residents of Woods Hole will be glad to hear that the Tennis Club has started its yearly activities. The courts are all in good playing order, and it is urged that everyone take note of the extensive repairs which have been made on backstops, nets and playing surfaces.

The membership fee for this season is $4.00. Junior membership (for all those under sixteen) is $2.00. All dues are payable to Dr. Phil B. Armstrong.

A North Carolina blacksnake in Dr. R. M. Stabler's laboratory is in the process of laying eggs as the *Log* goes to press. The eggs have soft shells, and are white, a little over an inch long and a quarter of an inch high, much more flattened than hens' eggs. Dr. Stabler expects to hatch the eggs and add seven more blacksnakes to his laboratory collection, which includes the blacksnake, a garter snake, and a six-foot gophersnake from Florida. A rattler may arrive later this summer.

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TRANSMISSION OF NEUROHUMORAL SUBSTANCES

DR. G. H. PARKER
Professor of Zoology, Harvard University

The subject of my talk tonight is something that grew out of a paper presented last summer and is a continuation of some work begun a number of years ago. It concerns the transfer of substances in the body, the so-called neurohumoral substances which are probably produced by the nervous system and may affect local as well as distant parts of the body. As far as fluid exchanges in the body are concerned, we ordinarily think simply of blood and lymph. Blood carries food, oxygen, and hormones and when it reaches the capillaries, the fluids pass through the walls and in this way the lymph thus formed reaches the cells. The cells take up the nutriment and these living units give out excretory products which are picked up again by the lymph carried to the blood, and thus the circulation goes on.

There is evidence, however, of transmission of substances from cell (*Continued on Page* 62)

M. B. L. Calendar

TUESDAY, JULY 18, 8:00 P. M.

Seminar: Dr. G. W. Kidder: "Chromatin Extrusion in Certain Ciliate Commensals of Mussels."

Dr. F. M. Summers: "The Reorganization Bands in the Macronucleus of Aspidisca."

Mr. H. E. Finley: "Comparative Studies on the Osmiophilic and Neutral-red-stainable Inclusions of the Genus Vorticella."

Mr. W. L. Doyle: "Experimental Cytology of Amoeba Proteus."

FRIDAY, JULY 21, 8:00 P. M.

Lecture: Dr. Balduin Lucke: "The Zoological Distribution of Tumors."

Members of the M. B. L. Club (and those planning to join) are urged to come to the Clubhouse immediately after the lecture to attend the first weekly smoker.

THE BERMUDA BIOLOGICAL STATION FOR RESEARCH

PROFESSOR EDWIN G. CONKLIN
President of the Corporation

The Bermuda Biological Station for Research, Inc., is one of the newest of the biological stations as the Marine Biological Laboratory is one of the oldest. In its organization the officers have had the advantage of the experience of many years at the Woods Hole laboratory and it is hoped that an account of the Bermuda Station may be of interest to the readers of THE COLLECTING NET. The Station is now located in one of the most beautiful sites in Bermuda, and has thoroughly adequate buildings and laboratories. It is the only station in the west Atlantic that is open to receive research workers every month of the year, and an increasing number of investigators will visit there throughout the winter months as well as during the summer.

No courses of instruction are offered at the Bermuda Station, since it is exclusively a research institution. Investigators in any field of

TABLE OF CONTENTS

SHORE HILLS FROM SOUTHEAST
Showing Verandas and Sleeping Porches

biology or oceanography are welcomed and are given all the facilities possible for carrying on their work. The regular charges for research room or table are $400 per year, $100 for three months, $25 for two weeks or less, but the president or director is authorized to remit these charges in whole or in part in special cases. It is hoped that many of the investigators at the Woods Hole laboratory may find it possible to utilize the facilities offered at the Bermuda Biological Station.

The history of the Station from its inception down to the beginning of the year, 1933, is contained in the following statements taken from the reports of the president and director, which are soon to appear in the Annual Reports of the officers of the Station.

The Bermuda Biological Station for Research was established in 1903 with the cooperation of the Bermuda Natural History Society, Harvard University and New York University, with Professor Edward L. Mark of Harvard as director, and Professor Charles L. Bristol of New York University as associate director. For three summer sessions the work of the Station was carried on in buildings of the Hotel Frascati, near the present Government Aquarium. In 1907 the Bermuda Natural History Society leased from the War Department Agar's Island, converted its "magazine" into a public aquarium and invited Professor Mark to continue the sessions of the Biological Station there. Summer sessions were held there from 1907 to 1930 inclusive, with the exception of the years 1917-18 when Agar's Island was requisitioned for military purposes and the Station was transferred to an island near by. During all this time Dr. Mark served as director and in the years 1915-18 Dr. William J. Crozier was resident naturalist and the Station was kept open throughout those years. In the 28 years of the original station about 280 scientists were in attendance and nearly 170 articles were published as the result of work done there; these articles have been assembled by Dr. Mark in seven volumes of "Contributions from the Bermuda Biological Station for Research."

Plans for the reorganization of the Station on a broader and more permanent basis had their inception at a meeting of interested persons at Woods Hole, Massachusetts, in August, 1925. As an outcome of that meeting it was decided to follow in the main the plan of organization which had been so successful at the Marine Biological

Laboratory and also at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, namely, the association into a Corporation of a large number of persons who were interested in the Station, and the subsequent election by this Corporation of a Board of Trustees to administer the affairs of the Corporation. This plan received the endorsement of the National Research Council of the United States, the Royal Society of London, the Royal Society of Edinburgh, the Royal Society of Canada, the Biological Board of Canada, and the Honorary Council for Research (Canada). One hundred and eighty persons joined the Corporation and a Committee on Reorganization was elected. This Committee drew up articles of incorporation and by-laws and nominated sixteen members of the Corporation to be trustees, the twelve receiving the highest number of votes to be declared elected. By the votes of more than 150 members of the Corporation twelve trustees were elected. On June 28, 1926, the Bermuda Biological Station for Research was incorporated under the laws of the State of New York, and on April 26, 1930, the articles of incorporation were amended to permit the enlargement of the Board of Trustees to twenty-four. A more complete account of this period in the history of the Station was published in *Nature*, Jan. 22, 1927, and in *Science*, Feb. 4, 1927.

Special meetings of the Trustees were held in August and October, 1926, and the first annual meeting of the Corporation and Trustees was held in New York, December 27, 1926. At that meeting offcers were elected, an Executive Committee appointed and a report received from a committee of four Trustees who had visited Bermuda to select a site for a permanent station. After this committee had inspected more than twenty proposed sites they reported in favor of a tract of 12 acres in St. George's Parish, known as the "Hunter Tract." This report was approved by the Trustees and Corporation and later a petition was addressed to the Governor and Legislature of Bermuda asking (1) that the Bermuda Biological Station for Research, Incorporated, be granted the privilege of holding real estate in the Islands of Bermuda; (2) that when the Trustees should satisfy the Governor-in-Council that not less than £50,000 endowment had been raised the Colonial Government should purchase and transfer to the Trustees the Hunter property; (3) that all supplies and equipment imported for the purposes of the Station be exempted from customs duties; (4) that an annual grant of £200 for a period of ten years be made by the Bermuda Government for the support of the Station. On June 24, 1927, "The Biological Station Act, 1927" was approved, enacting each and all of these articles.

The Trustees then applied to the General Education Board of the Rockefeller Foundation for a grant to meet this conditional gift of the Bermuda Government and to provide for the development and maintenance of the Station, and on November 13, 1929 the Rockefeller Foundation appropriated £50,000 for the purpose. On March 29, 1930 the Hunter tract was purchased by the Government of Bermuda and conveyed to the Trustees, and on April 4, 1930 the Rockefeller Foundation paid to the Trustees £50,000 ($243,265.63).

After plans had been prepared for a laboratory building but before construction had been started, the President and Treasurer of the Station were offered another near-by property known as the "Shore Hills Hotel and Sanitarium," consisting of fourteen acres of land, a large hotel building and several smaller buildings, with pumping station, jetties, bath houses and well-kept grounds for a price which would involve a large saving as compared with the cost of developing the Hunter tract. Some leading citizens of Bermuda suggested that the Trustees reconvey the Hunter tract to the Government on condition that the latter pay to the Trustees its purchase price of £5,500 to be used in acquiring the "Shore Hills" property and converting it to the uses of the Station. On August 13, 1930 the Executive Committee at a meeting in Woods Hole approved this proposal, provided that the Legislature of Bermuda would arrange for the exchange, that the Rockefeller Foundation would consent to it, and that this action be approved and ratified by a majority of the entire Board of Trustees.

Maps and photographs of the Shore Hills property and blue prints of the main building, showing the proposed alterations, together with blue prints of the proposed new laboratory on the Hunter tract with a comparative statement of the advantages, disadvantages and estimated costs of each, were prepared by the President and submitted to the Trustees, and in September 1930 they voted almost unanimously in favor of exchanging the Hunter tract for the Shore Hills property, subject to the conditions specified by the Executive Committee.

As a result of further expert examination of the Shore Hills buildings and more detailed estimates of the cost of repairs the Executive Committee on November 29, 1930 asked the owners to reduce their price for the property. On December 24th the purchase price was satisfactorily adjusted and steps were taken to secure the consent of the Rockefeller Foundation and of the Bermuda Government to the transfer from the Hunter tract to Shore Hills.

At the annual meeting in New York on January 3, 1931 it was voted "that the Trustees approve President Conklin's action in offering $75,000 for the land and buildings of the Shore Hills Hotel and an additional $5,000 for the furnishings, it being understood that the offer is conditional upon the Bermuda Government's being willing to resume ownership of the Hunter Tract and to pay £5,500 toward the purchase of the Shore Hills property." This plan was submitted to the Rockefeller Foundation since it had paid to the Trustees of the Station £50,000 to meet the conditional grant of £5,500 by the Bermuda Government for the purchase of the Hunter Tract, and on January 14, 1931 this exchange of property was approved by the Rockefeller Foundation.

The "Biological Station Act, 1931" authorizing the payment of £5,500 to the Trustees of the Station, when the Trustees shall reconvey to the Bermuda Government the Hunter Tract, was passed by the Legislature and signed by the Governor of Bermuda on March 14th, and on March 18th the Hunter Tract was reconveyed to the Government of Bermuda and soon thereafter the sum of £5,500 was paid to our agent in Bermuda. On March 26th the President and Treasurer of the Station received the deed to the Shore Hills property, and paid to the former owners out of uninvested funds $80,000.

A description of the new Station and announcement of the first session from June 15th to August 10th was published in the *Scientific Monthly* for June, 1931.

A mess was opened in the main building. During the first session twenty workers and guests were accomodated at approximately actual costs, which were $15 a week for room and board, just one-half the charge at the Grasmere Hotel for workers at the old Station on Agar's Island. Arrangements have been made for accomodating workers and guests at the Station at any time throughout the year at this price when the mess includes six or more, and at $16 a week when less than six are present. Hereafter the Station will be open to workers throughout the entire year, and persons desiring to use its facilities should make application on a blank form which may be obtained from any of the officers or trustees.

The list of workers and guests at the Station during the first session follows: Dr. T. C. Barnes, Yale; Dr. N. J. Berrill, McGill; Mrs. N. J. Berrill; Dr. R. E. Bowen, Harvard; Mrs. R. E. Bowen; Dr. E. G. Conklin, Princeton; Mr. J. K. Donahue, Princeton; Miss Olive Earle, Artist, N. Y. City; Miss Jean Henderson, McGill; Dr. H. S. Hopkins, N. Y. University; Mr. C. M. Lee, Jr., Univ. of Virginia; Dr. A. W. Lindsey, Denison University; Mrs. A. W. Lindsey; Mr. David Lloyd, McGill; Dr. E. L. Mark, Harvard; Dr. C. M. McFall, Univ. of Virginia; Dr. G. E. Nelson, Col. City of New York; Mrs. Nelson; Dr. G. G. Scott, Col. City of New York; Dr. C. M. Yonge, Plymouth Lab., England.

The Board of Trustees, at its meeting in Woods Hole on August 12, 1931, considered carefully the question of the appointment of a resident director. It was felt very important that we should have a director on the ground as soon as possible, and after careful consideration of possible candidates and of the present budget situation, it was decided to offer the directorship to Dr. John Francis George Wheeler, age 32, B.Sc. and Sc.D. of the University of Bristol, Student at the Marine Biological Laboratory at Plymouth. Investigator for the Ministry of Agriculture and Fisheries, Zoologist on the "Discovery" Expedition 1924-'27 and 1929-'30, for the past year in charge of the scientific office of the "Discovery II" in London and designated for the scientific leadership of the "Discovery II" on her next commission. Dr. Wheeler accepted our offer and he and his wife have been in residence at the Station since January 1st, 1932.

A grant of $2,000 a year from the Woods Hole Oceanographic Institution is a very welcome addition to our income and is made as payment for the use of the Station and its facilities by members of the Woods Hole Oceanographic Institution; this use will be chiefly through the winter months when there are likely to be fewer visiting scientists at the Station than during the long vacation in summer.

On November 13, 1931, the Executive Committee of the Rockefeller Foundation appropriated the sum of $12,000 to the Bermuda Biological Station for Research, payable at the rate of $6,000 a year over a two-year period beginning January 1, 1932.

Specific needs which can be met by the generous cooperation of members of our Corporation and Board of Trustees are for microscopes, microtomes, physiological and chemical apparatus, books, journals and reprints. Almost anything of this sort would be welcomed if in usuable condition.

The year 1932 began with the formal opening of the Station and the public induction of Dr. Wheeler into the office of Director on January 6th. His Excellency, the Governor of Bermuda, presided on this occasion and his presence as well as that of members of his family and his Official Staff lent especial dignity to the occasion. Six members of our Board of Trustees and about five hundred invited guests were present, and addresses were made by His Excellency, the Governor, and by Mr. F. G. Gosling, Dr. Mark, Dr.

Conklin and Dr. Wheeler. An account of these exercises was published in *Science* for January 29, 1932, and reprints were sent to members of the Corporation and Trustees, as well as to many others interested in the Station.

The continued interest and support of the Government of Bermuda is gratefully acknowledged; its annual grant of £200 has been continued and free entry has been given for all apparatus and equipment, pilotage and port charges have been remitted for the research ship "Atlantis" of the Woods Hole Oceanographic Institution as well as for any other ship engaged in scientific work exclusively; in many other respects officers of the Government, notably the Director of Agriculture, the Director of Public Works, the Superintendent of the Bermuda Aquarium, and other citizens of Bermuda have been very helpful in promoting the interests of the Station.

The U. S. Coast and Geodetic Survey has donated to the Station a Standard Automatic Tide Gauge, with the understanding that it be suitably installed, together with certain bench marks, and that when in operation the tide roll be changed after each calendar month of record and forwarded to the Survey, where it would be tabulated and photostat copies would be furnished to our Station, to the Meteorological Station recently established at St. George's, and to such others as we may designate. Our best thanks are exxtended to these governmental agencies for their cooperation.

In view of the economic depression throughout the world no serious attempt has been made to increase our endowment or our regular income from cooperating institutions, although this must be undertaken at the earliest appropriate time. There has been no default of interest on our investments and this income, together with the emergency grant of $6,000 from the Rockefeller Foundation and the grant of $2,000 from the Woods Hole Oceanographic Institution have enabled us to close the year with a safe operating balance to our credit.

During the past year five institutions have subscribed to the support of one or more research tables at the Station, viz., Harvard, Yale, Princeton, The Academy of Natural Sciences of Philadelphia, and the New York Zooligical Society. Several other institutions have agreed to support tables but have not done so as yet. It is highly desirable that we should enlarge the list of cooperating institutions that make annual grants for the support of tables, whether they send representatives or not. In consideration of such continuing support the Station might well agree to accept more than one representative from each of the cooperating institutions, as long as our facilities permit. We gratefully acknowledge the receipt of $500 from Mrs. J. J. Storrow, which gift has been used to provide a new launch to replace the old one which was unseaworthy. In addition to subscriptions of cooperating institutions four workers have contributed personally toward the support of their research tables.

Scientific cooperation on the part of institutions is no less important than financial support and here success may depend to a considerable extent on scholarships or other forms of aid for worthy and needy investigators. Some excellent research men were unable to work at the Station this past year because of lack of funds to pay traveling and living expenses. The Station has accepted all qualified applicants even if they were unable to pay for their research tables, but it cannot afford to give free room and board, unless in return for services rendered. A system of scholarships or fellowships which would pay the living and traveling expenses of excellent workers who could not otherwise come to the Station would be very useful. The Tortugas Laboratory of the Carnegie Institution of Washington makes such provision for all who are invited to work there. Some of our cooperating institutions provide funds to pay in whole or in part the living and traveling expenses to their representatives at the Bermuda Station, in addition to the rent of a research table, and it would be well if all of them would do this. As soon as possible the Station should set aside a sum of say $1,000 a year to help pay the expenses of selected persons who would be invited to join the staff and carry on research at the Station for a limited period each year. Approximately twenty experienced investigators volunteer to serve on the staff at the Woods Hole Marine Biological Laboratory without pay, receiving at most free research rooms. It would be an excellent move for the Bermuda Station to make a similar arrangement with several experienced investigators, even at the additional cost of supplying some of them with free living accommodations. No more economic method of furthering the scientific work of the Station could be devised than one which would thus bring to the Station and add to our staff at so small a cost investigators who would further the scientific work of the Station. For some time to come we shall need to depend largely on volunteer workers and it would be highly advantageous to have on hand a staff of such workers who could be counted on to help maintain the scientific *esprit de corps*.

Indeed one of our most important needs at present is that the scientific world should become better acquainted with the Bermuda Station and that more students and investigators should make use of its facilities. The Station could readily

accommodate 25 or 30 persons at one time. The Station is open throughout the entire year, but there are many months during the usual academic year when very few if any visiting workers are in residence. If the Corporation and Trustees were to make known to scientists generally, and not merely to biologists that the Station would welcome all scientists who could make use of its facilities, it would help to make the Station more widely useful. It must not be forgotten that the financial as well as the scientific success of the Station will be greatly influenced by the extent to which it is used. In this connection it is highly important that the living conditions and social atmosphere of the Station should be as agreeable as possible and that our charges to workers should be kept at the lowest figure practicable.

The Trustees have decided that it is permissible and desirable, when facilities for room and board are available, to take in certain classes of paying guests, who are not actually doing scientific work, charging them a higher price than in the case of workers, and that cottages that are not otherwise assigned might be rented to such persons so as to augment the income of the Station. If desirable people can be thus attracted to the Station it will be a contribution to its social life as well as to its income. Of course such an arrangement should never be permitted to interfere with the scientific work of the Station, but with the excess rooms and cottages available, especially during the winter, it may well become a valuable source of income.

Chief among our material needs is an ocean-going motor-boat with auxiliary sail, equipped with engine, power winch and wire rope, nets, thermometers, sampling bottles, refrigerator, etc., for work in deep water outside the reefs and around the islands. Plans and specifications for such a boat were carefully prepared after consultation with the Directors of the Bermuda Aquarium, the Woods Hole Oceanographic Institution, the Plymouth Laboratory and many others. Estimates were obtained from several boat builders and agencies for the sale of second-hand boats; some of these estimates are listed herewith.

a. Fisherman's type of boat, 40 ft. long, 12½ ft. beam 4 ft. draft, 40 h.p. gasoline engine, $4,000-$5,000. Built to specifications for Bermuda conditions $1,000 more.

b. Larger boat built to specifications, 50 ft. long, 13½ ft. beam, 5 ft. draft, 40 h.p. gasoline engine $7,000. Or 60 h.p. Diesel engine $8,000.

c. Type of boat recommended by Ralph S. McCallan, who is especially well acquainted with Bermuda conditions and the Station's needs, 55 ft. long, 15 ft. beam, 5 ft. draft, 60 h.p. Diesel engine $9,000-$10,000.

d. Extra strong steel winch connected with engine $950-$1,000.

e. About 1 mile of ¼ in. steel rope, and 3 miles of 3-16 in. steel rope, probably not less than $1,000.

f. Scientific and other equipment ca. $1,000.

Total approximate cost of smaller boat $7,000-$10,000. Larger boat (McCallan's type) $12,000-$13,000.

In making so large an outlay as is represented by any of these suggestions we should not be satisfied with makeshifts, but should seek to obtain a boat that will meet all our needs for many years to come. The cost of keeping such a boat in commission with a captain, a deckhand or boy, fuel, repairs and insurance would probably not be less than $2,500 a year and might be much more.

(Continued Next Issue)

TRANSMISSION OF NEUROHUMORAL SUBSTANCES

(Continued from Page 57)

to cell even in complicated animals, which is quite independent of other fluids circulating in the body. I became interested in this through a study of the chromatophores, and it is their response that I wish to call to your attention. The flat fishes are useful examples since they vary in color from light to dark with the background. Dr. Mast in his paper in Bull. U. S. Bur. Fish. shows the remarkable capacity for change in the exterior aspect of these fishes by the chromatophores. Pouchet has shown very interesting responses in turbots. These changes depend more or less on the nerves. If a nerve is cut the region does not change markedly at first but it takes place very slowly as a whole. In general I believe that these changes were connected with the nerves. When the eyes were covered the whole system failed to act; when one eye was left uncovered, however, it carried on just the same. In frogs, it does not matter how much you cut the nerves; the color is not influenced. These animals change over the whole body rather than in parts of it. The pituitary gland is responsible in this case. When the stimulation is strong, the change is great; when the stimulation is light, the animal quickly relapses

to its original condition. It is interesting to contrast the reactions occurring from cutting nerves in flat fish and the color changes in frogs, the one connected with nerve secretions and the other with gland secretions.

The experiments of Hogben and Mirvish on chameleons show that the part in which the animal fails to make any change is the part that is brought out by cutting the spinal cord. When the cut is made at various levels the animal fails to change color posterior to the cut. The condition in these reproduces the condition in fish. It is quite evident that the nerves are the significant point. Last summer I called attention to the fact of these contrasts—a nerve control in flat fishes, hormones in frogs, and in reptiles nerves again. My explanation is that we were really dealing with one state of affairs manifested in these various ways. In all cases the chromatophores respond to nerve secretions. In fishes the creature responds to a local secretion at the ends of the nerve fibers, causing the chromatophores to contract or expand. In frogs, on the other hand, the secretion comes from a local gland, is given out and passes through the blood a long distance to affect remote parts of the body. In fishes and reptiles the secretion is poured out at the tips of the nerves and there is a local reaction, although it may spread to give a general reaction. There is not a difference in principle but different applications of the same general principle of nerve secretion. This secretory explanation has been suggested by Sherrington, Gerard, Giersberg, Koller, Meyer, Parkêr, and Perkins and Kropp.

This phenomena operates only in cold-blooded vertebrates. The higher vertebrates have no such systems that we are aware of—they change color by changing the hair or feathers. We have studied in particular the condition in Fundulus tails. If you place Fundulus in a light dish, in five or ten minutes they are quite light, or if put in a dark environment, they become dark, the changes taking place quite rapidly. Examination of the animals under these conditions indicates the mechanism that brings this about. For example, we have the fin rays with the chromatophores in the light state, that is, they are reduced to the size of a dot, with the color material in the center of the cell. When the tail is in the dark state, the cells have sent the pigment out into their processes, and the chromatophores are then in a broad and extended condition. There is obvious cellular response produced by so-called expansion and contraction of the cells. The material is permanent and migrates out when the chromatophores are in an extended state.

If in an ordinary Fundulus you make a transverse cut in the tail 1 or 2 mm. in length, at the end of thirty seconds a dark band appears across the tail. The nerve fibers in the cut region excite the cells and the dark area is formed in the otherwise light fish. We have studied this tail stripe with a good deal of care. In an hour or two the band reaches its maximum intensity, and then fades out in a day or two. At first there are fairly sharp edges then they gradually fade. This is explained by the assumption that substances are produced which bring about these conditions. In cutting, the nerve fibers are stimulated in regions lying beyond the cut. When this stimulation dies down, there is no longer any differentiation. The nerve degenerates in 6 or 8 days, but in addition the reaction is probably influenced by introduction of material from the fibers into the band. "Contracting" material could creep in at the edges, gradually causing the cells to contract, that is, they contract not simply because the nerves cease to act, but because a neurohumoral substance comes in from the fibers outside the streak and causes the melanophores to change. Evidence for that can be seen. The band does not disappear as a whole, but it begins at the periphery and changes progressively. This leads me to conclude that the process is one in which something percolates in from the outside producing the contraction of the melanophores.

I should like to say a few words about the details of this work. Last year one of my students, Miss Mills, worked upon this portion of the fish and came upon a very interesting point. She was particularly interested in studying the edge of the band. She discovered that the edges of the dark and light bands did not coincide when produced on the tail of the same fish. I am unable to explain this disagreement between the edges of the two bands unless there be two sets of nerve fibers. One set spreads across from one point, and the other from another. The melanophores have different innervation, one set of nerves concerned with contraction and the other with expansion, that is, there is a contracting and an expanding substance acting alternately on the chromatophores. It is not always possible to follow one initial cut, but these conditions can be produced in a recurrent way. If you cut a fish in the beginning and allow it to bleach out in a light dish, and then put it in a dark environment, the whole fish quickly becomes dark except the band. After a few hours the band, too, becomes dark. The same thing occurs when you put a dark fish in a light environment. This lagging-behind exhibited by the band region can be explained again by the percolation of substances into the denervated region causing changes in the cells.

There is further evidence in this later reaction to favor the idea that neurohumoral substances

are active here. An initial stripe of 1 mm. disappears in 22—96 hours, these figures being minimum and maximum time limits. The average for 25 animals is about 29 hours. A 2 mm. stripe requires 34—143 hours, with an average of 78 hours for complete disappearance; the time for disappearance therefore varies in proportion to the size of the initial stripe. It is a remarkable fact that if you take a fish in the dark condition, dark body and dark band, and inject adrenalin, the whole animal becomes light in ten to fifteen minutes. This, of course, is a question of change in blood and lymph which determines a response over the whole body. Neurohumoral changes, on the other hand, take place much more slowly. The substances are perhaps oil soluble and pass through the lipoid coverings of the cells from one to the other.

What do we already know about cell transfers? There are a great many examples in cells in plants, for instance. Within the last year Kok published a paper on the transfer of caffeine through the tentacles of Drosera. These were immersed in a weak solution of caffeine which makes its way into the cells and produces a slight precipitate so that its progress can be detected. It can be seen to pass transversely through the cells at a very slow rate. It was estimated that the distal spread was at a rate of about 658 micra in 30 minutes, and the proximal, about 534 micra in 30 minutes. The spread in Fundulus is very much slower—9 micra in 30 minutes.

Examples of this spreading of substances can be found in the human skin. Sir Thomas Lewis showed very striking evidence of it in his experiments with irradiating skin with ultra-violet. The skin of an arm was partly covered with an impermeable substance so that the reddening appeared in a definite pattern. In the course of a day the edges of this reddening become less distinct and spread out latterly to a distance of 2-3 mm., or if the treatment is strong, 4-5 mm. beyond the pattern. Another instance can be found in erysipeloids, or sores to which fishermen and poultry men are peculiarly susceptible, and which appear isolated at first and then slowly spread to surrounding areas. Insect stings often react similarly, and the "piebald" skin that appears sometimes in Caucasians and negroes is a further example. The latter consists of a loss of pigment which gradually spreads throughout the surface of the skin, the afflicted individual remaining otherwise healthy and strong. It seems likely that this change is comparable to that occurring in the melanophores.

There are naturally a great many examples in the lower animals. In Coelenterates it is impossible to account for the transmission of nutriment from the digestive tract through the endoderm cells to the outside ectoderm cells, except by some such means as the passage of substances from cell to cell. It may be said that this manner of transmission is a much more primitive one than that by blood and lymph; it is found in all plants and animals from the lowest to the highest. It is probably operative in embryonic and regenerative tissue and in processes which are relatively slow.

SOME PRELIMINARY DATA FROM A CORRELATED ANATOMICAL, PHYSIOLOGICAL AND BEHAVIORISTIC STUDY OF THE REPRODUCTIVE CYCLE IN THE FEMALE GUINEA PIG

Dr. William C. Young

Assistant Professor of Biology, Brown University

Recent investigations on the oestrous cycle in our domestic and laboratory mammals have dealt very largely with special aspects of the subject, such as the cyclic changes in the genital tract, causal factors involved in the general phenomenon of oestrus, and to a lesser extent, with the rhythmic changes in behavior. There has been lacking, however, any comprehensive correlation of behavioristic changes with structural changes. For this reason, such a study has been undertaken and, because of the striking oestrous behavior manifested by the guinea pig, this animal has been used.

With the help of Mr. Hugh I. Myers and Mr. Edward W. Dempsey, in conjunction with whom the investigation is being conducted, the normal behavior of ninety females was observed continuously over a period of more than two and a half months. During each pro-oestrum, oestrus and metoestrum, the animals were examined at half hourly intervals and data were recorded with respect to the condition of the vaginal closure membrane, the behavior of the females in the cages, and the assumption of the copulatory position when touched. During the period of observation we were able to make 17 series of vaginal smears at half hourly intervals from the time of the first opening of the vaginal closure membrane

until its closure. We were able to determine accurately, on the basis of 231 individual cycles, the length of the oestrous or reproductive cycle and its variation in length during successive cycles. We were able to ascertain the length of the oestrous period, that is, the period of sexual receptivity, by observing our animals continuously during 343 oestrous periods. This manner of observation enabled us to ascertain the variation in length of successive oestrous periods in the same animal. We were able to determine the time of day when oestrus is most likely to begin and the time when most animals are likely to be found in heat simultaneously. Lastly, we were able to determine the sequence of events which characterize oestrous behavior.

After this systematic study of the oestrous behavior in normal females had been completed, additional data which would involve the sacrifice of the animals were obtained. At this time we undertook to make a study of the time of follicular development, to determine the number of follicles which develop and rupture at each oestrus, to correlate this number with the length of the oestrus, to determine the exact time of ovulation and to relate it to the other events of the cycle and period, to determine the effect of copulation upon the length of oestrus and the time of ovulation, and finally, to determine the fertility of the female when mated at different times during the oestrous period.

Finally, seventy-six animals were killed at twelve different stages in the established oestrous cycle and from each animal we removed and preserved both ovaries, a section of one uterine horn, a section of the vagina, one mammary gland, and the hypophysis in addition to making a vaginal smear.

It has not yet been possible to analyze all the data that have accumulated or to study all the material histologically. On the other hand, many of our data are complete and it is these which are being reported.

It was learned, first of all, that the mean length of the oestrous period is almost exactly 8 hours. The range, however, was considerable. Two animals which appeared perfectly normal in every other respect never did come into heat even though the vaginal closure membrane ruptured regularly. With the exception of these two animals and an occasional "miss," the shortest period was one of an hour's duration, the longest 14.5 hours.

One of the most definite events of the oestrous period is the time of ovulation. As nearly as could be ascertained from an examination of forty-two animals, ovulation occurs within an hour and a half of the end of oestrus whether oestrus lasted three, four or five hours, or twelve, thirteen or fourteen hours.

From an examination of fifty animals it was found that no relationship exists between the length of oestrus and the number of developing follicles. Animals in which one follicle had developed remained in heat 7 to 11½ hours; animals in which four follicles had developed remained in heat approximately the same time or 5½ to 10½ hours.

One of the most interesting characteristics of the oestrous period is the frequency with which oestrus occurs at night rather than during the daylight hours. Data have accumulated from 442 observations. Actually two-thirds of all the animals came into heat between 6 P. M. and 6 A. M., while a large part of the remaining one-third came into heat between 4 P. M. and 6 P. M. A minor peak of activity at 6 P. M. was at first puzzling. However, after curves had been prepared from data for the two halves of the experimental period, February 15 through March 31, and April 1 through May 10, a possible explanation became clear. It was found, first, that animals show an especial tendency to come into heat during either of two periods in the day, late in the afternoon or early in the evening, or if not then, later in the evening between about ten and two; secondly, that these maxima of oestrous activity shifted to the right as the days became longer, that is, toward the late night and early morning hours; and thirdly, that the hump to the left of the curve prepared for the entire period of observation is really a second maximum which expresses the frequency of oestrous activity in the late afternoon and early evening and which has become somewhat leveled off because of the shifting of the time of oestrous activity with the lengthening day.

Obviously, the occurrence of oestrus is subject to some nocturnal influence. What this influence can be is not understood. Nor are we able to explain why there should be two maxima of activity unless in many animals threshold changes preparatory for heat are partially built up during the last night of the dioestrum and completed early the following evening. Both problems are being investigated.

The entire study as far as we have progressed is, of course, preliminary. It has indicated, however, that oestrus is not yet an exhausted subject and that factors may be involved, the existence of which has not hitherto been suspected. The data which have just been presented may or may not suggest what these factors are, but they have suggested several interesting working hypotheses which have provided the basis for experiments which are now in progress.

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

Printed by the Darwin Press, New Bedford

THE M. B. L. CLUB

Investigators and students alike are indebted to the out-going president for reviving the important work of the M. B. L. Club. Handicapped by a broken leg, and relatively inefficient co-officers, he has served as a catalizer so efficient that Professor Robert Chambers has consented to serve as president for the present year; Dr. Heilbrunn has stirred numbers of the scientific community to appreciate the important part that the club can play in their lives at Woods Hole. His contribution has been great. The M. B. L. Club can do much to add to the pleasure of laboratory life. But it can do more than that. It can make a definite contribution to biological research. Gatherings in the club-house will serve as a medium for the interchange of ideas and act as a stimulus to the younger investigators.

More than one prominent biologist has expressed the opinion that they primarily benefit from the meetings of the American Society of Zoologists by the meeting old friends, the making of new ones, and by the consequent exchange of ideas. Zoologists have read, or can soon read, the substance of any paper on the program. This visual impression will mean more than the auditory one amidst many distractions. The situation at Woods Hole is analogous. The session here is not three days, but three months. The scientific program consists of the lectures and evening meetings. Extensive discussion is an important adjunct to the evening programs and it should be fostered. The absence of a suitable environment has prevented the members of the laboratory from taking full advantage of their opportunity. That is why the club's decision to hold a smoker after each Friday evening lecture is a fundamental one. As soon as the lecture is completed, the speaker and all members of the club are asked to adjourn to the club-house. Refreshments and "smokes" will be served and on cool evenings an open log fire will radiate its warmth about the "smokers". The gatherings will be "co-educational."

SEAL SCHOLARSHIPS

TO THE EDITOR:

Why cannot there be a COLLECTING NET Scholarship to make it possible for two seals who would not otherwise be able to come, to spend the summer in Woods Hole? Due to the depression or the mackerel or something the Fisheries people seem unable to furnish the animals. For a number of years the seals have been universal after dinner favorites and their contributions to the good humor and friendliness of Woods Hole have been great. They have won the respect and admiration of the most distinguished visitors. Hans Spemann was proud to climb into the seal pen, and be photographed with them, although he refused to be taken aboard the Cayadetta with members of the invertebrate course. Is it too much to hope that the COLLECTING NET will find it possible to tap its "many sources" and establish such a fund?

G. B. MOMENT.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M:
July 15	11:24	....
July 16	12:06	12:15
July 17	1:03	1:09
July 18	1:58	2:03
July 19	2:47	2:49
July 20	3:33	3:35
July 21	4:15	4:19
July 22	4:56	5:03
July 23	5:32	5:41
July 24	6:12	6:23

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

ITEMS OF INTEREST

Dr. H. Boschma, professor of zoology and director of the zoological laboratory at the University of Leyden in Holland, visited at the Oceanographic Institution over last week-end. He came to this country to attend the Pacific Science Congress in Victoria, Canada, and is sailing for Holland the fifteenth on the *Berengaria*. Dr. Boschma is not a stranger to Woods Hole having spent six weeks in 1924 at the Marine Biological Laboratory working with the coral, Astrangia. A paper covering his work here was published in the *Biological Bulletin* for June, 1925, under the title "On the Color Changes in the Skin of the Lizard Ptychozoön homalocephalum." He expects an article on the Dutch marine laboratory to The Collecting Net later in season.

Professor P. H. Mitchell of Brown University, with his family is visiting the Pacific coast this summer. He will be at the Scripps Institution of Oceanography at La Jolla, at the Hopkins Laboratory at Pacific Grove, and at the University of Washington in Seattle, for brief periods of work upon measurements of the alkalinity of Pacific coastal waters. This will be a continuation of similar work done at the Woods Hole Oceanographic Institution last summer.

In a letter from Dr. Florence Peebles we learn that she attended the meetings of the American Association for the Advancement of Science in Chicago, and that she will visit her family in Virginia. She hopes later to spend a few days both in Woods Hole and in Salisbury Cove. Her address until August 1 will be: c/o Dr. Eliz. McLaughry, The Overlook, New Wilmington, Pa.

Dr. Esther Carpenter spent the past year at the Johns Hopkins Medical School as a research assistant to Dr. C. W. Metz of the Carnegie Institution of Washington. She will teach next year in the biology department of Albertus Magnus College in New Haven and continue with her research work at Yale University.

Dr. B. H. Willier (instructor, assistant professor, associate professor and full professor of zoology at the University of Chicago from 1920 to 1933) has accepted the headship of the department of biology at the University of Rochester.

Dr. Robert Payne Bigelow has been given the title of Professor Emeritus Professor of zoology and parisitology at the Massachusetts Institute of Technology.

THE COLLECTING NET SCHOLARSHIPS

Dr. E. C. Cole, director of the course in invertebrate zoology at the Marine Biological Laboratory recently made the following comments concerning The Collecting Net Scholarships:

The Collecting Net Scholarships have proved to be of real value in stimulating qualified students, and enabling them to devote an additional summer to study or investigation at the Marine Biological Laboratory. The courses may appropriately be considered as "feeders" from which the Laboratory will secure individuals fitted to become investigators. Any factor which favors this ideal must be considered desirable, and that is precisely the rôle played by these scholarships. Awards are made on the basis of sound training, excellent work, and real evidence of future promise as investigators.

As instructor in charge of the Invertebrate Zoology Course, I am very glad to certify to the importance of these scholarships. It is highly desirable that they be placed upon a permanent basis. Certainly a permanent endowment for this purpose is by no means impossible. The achievement of this aim would be well worth the time and money necessary.

In a card to Mr. McNaught, Dr. A. R. Moore writes that he and Mrs. Moore are "greatly enjoying the surroundings and the wonderful sea fauna" in Misaka, Japan.

Dr. Gardner B. Moment (who likes seals!) will return to Goucher College next year as instructor in biology.

Dr. George L. Clark of the Oceanographic Laboratory is occupying Dr. Mitchell's cottage in Woods Hole during the present summer.

Dr. Victor Hamburger, formerly of the University of Freiburg, arrived at the Marine Biological Laboratory. He is a Rockefeller Foundation Fellow from the University of Chicago.

First reports from the Washington University-Woods Hole expedition to the Tortugas are beginning to filter through. The first day of the arrival was marked by a casualty, as Paul Nicoll, unused to the mysteries of coral reefs, stepped on one and was laid up for a couple of weeks with a cut foot, which limited his capacity for amusement but apparently not for work.

DISTRIBUTION OF THE FRESHWATER ALGAE OF NEWFOUNDLAND

Dr. Wm. Randolph Taylor
Professor of Botany, University of Michigan

In 1926 a collection of freshwater algae was made by Mr. J. M. Fogg, Jr., and in 1929 another by Mr. Bayard Long on expeditions directed by Prof. M. L. Fernald; in 1929 Miss Belle Burr also collected several samples on Newfoundland.

The controlling features of the distribution of the flowering plants on Newfoundland as reported by Fernald may be generalized as follows: The rocks east of the Long Range Mountains and south of the Northern Peninsula tend to develop an acid soil; the crests and western slopes of the range are predominantly calcareous, with some serpentine areas. The island in general escaped the main Pleistocene continental ice sheet, but had a local ice mass over the eastern part; the western summits underwent no considerable glaciation and probably were in considerable degree actually free of ice. . At present the eastern and east central part of the island is cold and foggy under the influence of the arctic Labrador current, while the western slopes and shores are relatively warm and sunny though the deep mountain valleys do retain heavy accumulations of snow.

Many arctic, alpine and Cordilleran vascular plants appear in the flora of the western border strip; the soil was favorable to them and their colonies either antedated the Pleistocene ice and survived on unencumbered portions of the western ridge, or became established as it retreated. Many southern coastal plain (Carolinian) plants find northern limits in the south and southeast, because they are adapted to the sterile acid soil and reached that area by a land bridge after the Pleistocene ice disappeared. These two special classes of plants, and a number of endemic species, accent a flora the bulk of which is composed primarily of wide-ranging Boreal, and secondarily of Canadian-Alleghenian species, though it must be noted that the sterile soil appears to inhibit the growth of many species of these major elements which would otherwise be expected.

In attempting an interpretation of the distribution of the algae on Newfoundland, it was necessary to limit attention to the one large group which was known in sufficient detail to afford a basis for comparison with other countries, namely the Desmids, of which over 500 kinds are to be recorded. Of all other freshwater algae less than 200 are recorded (excluding diatoms). There may be selected from the 4 largest genera 130 species which fall into three groups: one with an arctic-alpine range, one wide-ranging but northern, and the third wide-ranging but southern. Comparing the individual station lists with these, we find that the last or southern-ranging types do not have a distinctive distribution among the Newfoundland stations studied. The northern-ranging types are particularly abundant about Bonne Bay part-way up the west coast, and drop off about Ingornechoix Bay, a more northern station. At this place we find the maximum concentration of arctic-alpine species, the number being more than five times as great as at the other localities.

Some confirmation of the distribution features may be secured by noting what species are found in one district to the exclusion of any other. In the Ingornechoix Bay district there is clearly a group of notable arctic-alpine species; in the southern district the proportion of wide-ranging types is very large, but in the residue we find a few notably southern types, including the two, *Micrasterias arcuata* and *M. expansa,* which first attracted attention to the Newfoundland study.

The distribution of the desmids, although more generalized as appropriate to the wider ranges which desmid species cover upon the earth, was found comparable to that of the flowering plants.

THE ROLE OF BACTERIA IN THE FORMATION OF NITRATE IN THE SEA

Dr. Selman A. Waksman and Dr. Cornelia L. Carey
Woods Hole Oceanographic Institution

No other phase of marine bacteriology has attracted as much attention and has aroused greater interest than the process of nitrate formation in the sea, with the possible exception of nitrate reduction. The formation of nitrate in the sea is usually considered as the final step in the transformation of nitrogen and as that form of nitrogen which is most readily available to the phytoplankton and other marine plants.

Several theories have been suggested to explain the origin of the nitrate in the sea, the most important of which are the following: (1) Nitrate does not originate in the sea itself, but is formed in land soils and brought into the sea by streams and by land drainage; nitrate may also be formed by electric discharges and thus introduced into the sea. This theory, originally proposed by Boussingault and Schlösing, has later found support in the work of Nathanson and Gran. (2) Nitrate is produced directly in the sea by bacteria which oxidize the ammonia formed in the decomposition of marine residues, first to nitrite and then to nitrate. This theory was first proposed by Vernon and by Brandt and later found support

in the investigation of Baur, Thomsen, Issachenco, Lipman and Harvey. (3) A third theory is that of photochemical oxidation of ammonia. This was propsed by Rao and Dhar for the oxidation of ammonia in solutions and in soils, in the presence of proper catalysts, and was applied recently by ZoBell to sea water.

The investigations reported in this paper have been limited to the study of the role of bacteria in the process of oxidation of ammonium compounds in the sea. The nature of the medium used for demonstrating the presence of these bacteria was found to be highly important. Because of the specific physiological nature of these organisms and the difficulty of obtaining satisfactory growth on artificial substrates, special attention was paid first of all to the composition of the medium which would be favorable for development of these bacteria. By the use of a proper medium, one could easily establish the following facts: (1) Sea water, especially in the upper layers of the sea, is practically free from nitrifying bacteria or contains only very few cells of such organisms. (2) Nitrifying bacteria are limited entirely to the sea bottom. The presence of bacteria capable of oxidizing ammonium salts to nitrite in both sandy bottoms and mud bottoms could easily be established. In the case of the latter, these bacteria are limited largely to the very surface layers of the marine bottom. The oxidation of the nitrite to nitrate in the cultures could be demonstrated only after a long period of incubation, after all the ammonia has been oxidized to nitrite.

Although these investigations have been limited to the material obtained from various stations on George's Bank and in the Gulf of Maine, they lead to the conclusion that nitrate is produced in the sea in the bottom by the action of specific bacteria; the nitrate then diffuses from the bottom upward into the water.

DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION

By GEORGE L. CLARKE
Instructor of General Physiology, Harvard University

ABSTRACT

Observations on the vertical distribution of copepods were made in the Gulf of Maine during a 12-hr. period, a 24-hr. period, and a 48-hr. period. Careful records of the light falling on deck were kept during these periods and measurements of the penetration of light into the sea were made at frequent intervals using the photo-electric method. These simultaneous observations enabled quantitative information to be procured on the importance of light in controlling the diurnal migration of plankton.

Five closing nets were towed simultaneously at different depths from a single verticle cable. A method was devised for sending the nets down in the closed position, opening them simultaneously and towing them all together horizontally for 10 minutes, and then closing them again before hoisting to the surface. This method made it possible to sample accurately the upper 50 meters of water at intervals of less than one hour. Ordinarily ten series were made each day and in some cases the series was repeated in the 50 to 100 meter stratum.

The errors involved in the work at sea and in the sampling of the catch in the laboratory are at least as small as in other investigations of this type. Moreover, the methods used allow special dependence to be placed upon differences found among the hauls within each series.

The general vertical distribution of the three species studied was as follows: *Centropages typicus* inhabited the stratum of water above the thermocline (10 to 20 meters, *Calanus finmarchicus* was irregularly distributed, and *Metridia lucens* occurred below the thermocline.

The adult females of Metridia exhibited the most marked diurnal migration, the level of maximum abundance rising in the afternoon and during the night and falling in the morning. These movements were found to coincide to a considerable extent with changes in submarine irradiation. In the case of the 48-hr. station the behavior of these animals on the second day is very closely the same as on the first day. The changes in the vertical distribution of the other groups of copepods were slight or quite irregular. In some cases, however, there was a definite tendency for the maximum to occur at greater depths at noon than at other times.

This investiagtion confirms the idea that light is the most important factor controlling diurnal migration. In addition the observations are shown to have a bearing on various of the theories regarding the manner in which light exerts its effects. For example, the *change* of light intensity is shown to be probably not sufficiently rapid to reverse the sign of phototropism (as in Daphnia). Data on the rate of swimming of copepods indicate that these animals probably could keep pace with a given zone of light intensity as it changes its level during the course of the day.

THE BIOLOGICAL LABORATORY AT COLD SPRING HARBOR

Evening lectures at the Biological Laboratory thus far this summer have been given as follows:

June 23rd: Dr. Harold Abramson, College of Physicians and Surgeons: "ELECTROKINETIC POTENTIALS IN BIOLOGY AND MEDICINE".

June 28th: Dr. Charles B. Davenport, Director of the Department of Genetics, Carnegie Institution of Washington: "WHICH CAME FIRST IN EVOLUTION, FORM OR FUNCTION?"

July 6th: Dr. Felix Bernstein, of the Biological Laboratory: "THE CONNECTION BETWEEN PRESBYOPIA AND LENGTH OF LIFE."

July 11th: Dr. W. W. Swingle, Princeton University: "FUNCTIONAL STUDIES OF THE ADRENAL CORTEX."

Dr. Felix Bernstein, director of the Mathematical Institute of the University of Goettingen previous to the present political upset in Germany, is working at the Laboratory this summer under a special grant from the Rockefeller Foundation. His research is concerned with the relationship existing between weakening of the accommodating power of the lens of the eye and the length of life, and its inheritance.

Prof. F. Botazzi and Signora Botazzi, of Naples, were guests of Dr. and Mrs. Osterhout at Huntington before returning to Italy. Prof. and Mrs. Botazzi visited the Laboratory and were presented to those gathered for the symposium of July 5th on Oxidation-Reduction Potentials.

Two thousand five hundred dollars of a special grant from the Carnegie Corporation, to be used for equipment, has been received.

Dr. Harold Abramson, who is in residence at the Laboratory this summer, was very recently married to Miss Barbara Smith.

Dr. D. A. MacInnes, Dr. L. Michaelis, of the Rockefeller Institute, and Miss Elsa Michaelis, were guests of the Director of the Laboratory and Mrs. Harris on Tuesday and Wednesday of the week of the 3rd. Drs. MacInnes and Michaelis took part in the symposium of July 5th.

Other recent visitors to the Laboratory include Dr. L. R. Blinks, of the Rockefeller Institute, and Dr. E. S. Guzman Barron, of the University of Chicago.

DISTRIBUTION AND ECOLOGY OF THE MARINE ALGAE ON LAKE FISH

DR. H. P. BELL
Professor of Botany, Dalhousie University

The marine algae of the Atlantic coast of the Maritime Provinces of Canada were collected at representative places all along the coast. The most intensive collecting was done at St. Andrews, New Brunswick and at Halifax, Nova Scotia. A whole summer was spent collecting around Prince Edward Island. The report covers the work of more than seven years. The collecting was done chiefly during the summer, but regular collecting was also carried out for three winters. The species reported include thirty Chlorophyceae, forty-one Phaeophyceae, and forty-nine Rhodophyceae. The list of species, the regional distribution and the prevalence was given tabular form.

The coastal area of the Maritime Provinces is divided into three distinct geographical and ecological regions, namely, the Bay of Fundy, the Atlantic and the Prince Edward Island Regions. Each of these is distinctly different in regard to both marine flora and marine flora environment. The main features of the flora of each region are as follows: Bay of Fundy, generally dense and luxuriant; Prince Edward Island, a barren littoral zone and a rich sub-littoral floral; Atlantic, intermediate in density and luxuriance with the predominance of large linear forms in the surf. The dominant species are characteristic and constant for each region but they are quite different from region to region. The region that exhibits the greatest number of differences is that around Prince Edward Island. The physical factors varying throughout the area and associated with the floral differences are: water temperature, tides, wave action, clarity of the water as regards mud, structure and composition of the rocks along the shore, materials forming the ocean floor near the shore, slope of the region of growth, salinity and ice action. Each of these physical factors is associated with certain characteristic features of the marine flora. The growth is continuous throughout the year and consists of about five distinct crops. The barren period is in September or October and the most varied growth in March or April. The most important result of the survey was the demonstration of the wide differences existing between these three adjacent but sharply divided ecological regions.

. . . Announcement . . .

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In its office on Main Street THE COLLECTING NET has a great many books for sale. They cover a wide range of subjects and the prices of many of them have been cut to one half or one third of their original cost. Money resulting from the sale of these books will be used this summer to help defray the cost of publishing THE COLLECTING NET.

EPIDERMOPHYTOSIS (Athlete's Foot)

DR. DAVID CHEEVER
Harvard Medical School

(The following account is the first of two articles by Dr. Cheever throwing light on the possible danger of the spread of skin diseases through congestion of the Bay Shore bathing beach.)

"Athlete's Foot" has become very common in the last few years, particularly among people of cleanly habits, which is explained by the fact that such people are able to make greater use of gymnasiums, athletic clubs, bath-houses, and the like, where people tend to congregate bare-footed.

Bits of skin are constantly flaking off from our entire bodies and make up an appreciable part of the dust of our houses. From infected feet these particles of skin contain the germs of "athlete's foot", and in spite of ordinary care of floors, especially if their surfaces are rough, there is danger of foot infection.

Epidermophytosis is easily recognized on the webs of the toes, particularly between the fourth and fifth, and for some curious reason, more commonly on the left foot. It is in the form of macerated surface layers of skin, containing mycelium and spores, which can be readily rubbed off, dropping as tiny skin flakes to the floor to be picked up by the feet of passers-by.

Warm weather conditions make the skin more susceptible to infection by bacteria and fungi, and promote a more rapid and severe spread. Fortunately, there is an excellent check for those at the beach in the form of the extremely hot, dry sand. If the feet are wet for short periods only, and thoroughly baked in the hot sand during as much of the day as possible, a new infection may be kept from spreading and often a low-grade one may be largely, or quite, cleared up.

Advertisements in the current magazines to the contrary notwithstanding, there is no certain cure for "athlete's foot". No general rules for treatment, except sunlight and dryness, can be laid down because the infection varies so in different people and at different times. Most applications which are of any value in this condition are rather strong and liable to irritate so it is usually best to get competent medical advice for each individual case. In a general way, one may say that the cases limited to maceration between the toes may be safely treated with mercurochrome, sulphur ointments, ammoniated mercury ointment, but very cautiously with iodine unless it is freshly purchased since evaporation causes it to strengthen dangerously with age.

If the trouble becomes active and causes raw or blistered spots to develop about the toes, soles or sides of the feet, medical advice should be promptly sought as a pus infection (blood poisoning) occasionally enters, producing a great deal of soreness and temporary crippling. Epidermophytosis occurs not infrequently on other parts of the body, but no rules for its recognition or treatment can be simply given.

No entirely satisfactory methods of prevention have been devised, but around pools and shower-rooms various precautions are employed. In some instances all patrons are asked to wear rubber or paper shoes; in others, all are required to walk through a shallow tub containing sodium hyposulphite, or other solution; occasionally, all are requested to swab the webs of the toes with dilute iodine. In the home where a case of epidermophytosis exists, all members of the family should wear inexpensive paper shoes rather than to walk barefooted, and the patient's hose should be boiled each day.

NOTES FROM THE M. B. L. CLUB

At the annual meeting of the M. B. L. Club on Monday evening Dr. Robert Chambers was chosen president to succeed Dr. Louis V. Heilbrunn. Other officers elected at the same meeting were Dr. Samuel Shoup as Vice-President and Dr. Robert M. Stabler as Secretary-Treasurer. Miss Louise Mast was selected to serve as assistant Secretary-Treasurer.

The weekly M. B. L. Club dance will take place this evening at the clubhouse, beginning at 9:00 P. M. The attendance is expected to equal that of the first two, two-hundred or thereabouts.

A tentative program for next Wednesday's victrola concert, the second of the season includes Tschaikowsky's "Nutcracker Suite" in its entirety and the four movements of the First Symphony of Brahms.

The club is enlarging its facilities in line with its increased enrollment which is now one hundred and seventy, more than double that of last summer at the corresponding date. Beginning July 21, an informal smoker will be held after the Friday lectures.

In line with the recently-adopted policy of providing hosts and hostesses for each evening, the club has prepared the following list for the coming week:

Sunday, July 16—Mr. and Mrs. Norris Jones.

Monday, July 17—Reception for the new president and other officers—Dr. and Mrs. Robert Chambers.

Tuesday, July 18—Choral club rehearsal—Dr. Edwin Linton and Mrs. G. B. Jenkins.

Wednesday, July 19—Victrola Concert—Dr. and Mrs. P. B. Armstrong.

Thursday, July 20—Dr. Mary S. MacDougall.

Friday, July 21—Dr. and Mrs. G. B. Jenkins.

Saturday, July 22—Dance—Dr. and Mrs. R. M. Stabler.

THE COLLECTING NET

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SOME EFFECTS OF THE BLUE-GREEN ALGAE, APHANIZOMENON FLOS-AQUA, ON LAKE FISH

Dr. G. W. Prescott

Assistant Professor of Biology, Albion College

During the past three summers opportunity was afforded to study the efficiency of copper sulphate as an algacide and to study the various biological effects of superabundant growths of blue-green algae in some Iowa lakes. The investigations were carried on for the Iowa State Fish and Game Commission as part of a program to make the lakes suitable for the stocking of game fish.

Of late years there have been increasing periodic widespread deaths of fish in alarming numbers. The deaths have usually been associated with superabundant growths of blue-green algae. One of the many objectives of the investigation was to determine a possible casual relationship between the deaths of the fish and the algal condition of the lakes.

The most objection- (*Continued on Page 79*)

M. B. L. Calendar

TUESDAY, JULY 25, 8:00 P. M.

Seminar: H. B. Goodrich and C. B. Crampton: "One step in the development of hereditary pigmentation in the fish Oryzias latipes."

George D. Snell: "Translocations in the mouse and their effect on development."

D. E. Lancefield: "A series of probable mutations in Drosophila pseudo-obscura as compared with D. melanogaster."

P. W. Whiting: "Sex-determination in Hymenoptera."

FRIDAY, JULY 28, 8:00 P. M.

Lecture: Laurence Irving: "On the ability of mammals to survive without breathing."

Members of the M. B. L. Club are urged to attend the smoker following the lecture at the club.

ULTRACENTRIFUGAL AND CATAPHORETIC STUDIES ON RESPIRATORY PROTEINS

Dr. Theodor Svedberg

Professor of Physical Chemistry, University of Uppsala, Sweden

An ultracentrifugal study of the blood pigments—or respiratory proteins—throughout the animal kingdom has shown that these proteins are surprisingly well defined with regard to sedimentation constant and molecular weight. Not only the mass and shape but also the chemical composition of their molecules as revealed by the electrophoretic behavior shows uncommon distinctness. A detailed investigation of the different kinds of respiratory proteins is therefore of great interest not only from a physiological but also from a physico-chemical point of view. Three different properties have been studied: the sedimentation constant as derived from measurements of the rate of settling of the molecules in strong centrifugal fields, the molecular weight as calculated from sedimentation equilibrium determinations in cen-

TABLE OF CONTENTS

DR. ALFRED C. REDFIELD AND DR. THEODOR SVEDBERG

This photograph was taken just before Dr. Svedberg boarded the evening train for Boston at the conclusion of his stay in Woods Hole. He was a guest of the Woods Hole Oceanographic Institution and carried on his research work in Dr. Redfield's laboratory.

trifugal fields of medium strength and the isoelectric point as measured by means of the migration of molecules in electric fields.

The technique for the determination of sedimentation constant and molecular weight has been described in detail elsewhere. A small quantity of the solution enclosed in a sector-shaped cell is exposed to the influence of a strong centrifugal field in a special centrifugal instrument—the ultracentrifuge—and the concentration gradient determined by taking photographs of the solution during centrifuging. The pictures are then registered by means of a microphotometer and the curves obtained used for the calculations.

A refined technique for the study of the movement of the boundary between solution and solvent in a homogenious electric field makes it possible to determine the isoelectric point of the respiratory proteins with great precision. The measurements are made by taking photographs and registering the pictures in a microphotometer.

The main results may be summarized as follows. Respiratory proteins contained in blood corpuscles have always low sedimentation constants. Hemoglobin, characterized by the sedimentation constant 4.40×10^{-13} and the molecular weight 69,000, only occurs in the higher classes of the vertebrates. The blood corpuscles of the lowest class of the vertebrates, Cyclostomata, as well as the blood corpuscles of the capitellide worms have a respiratory protein of much lower sedimentation constant, $2.0\text{-}2.3 \times 10^{-13}$. The glyceride worms have corpuscle protein of sedimentation constant 3.5×10^{-13}, and probably a molecular weight equal to half that of hemoglobin. Respiratory proteins dissolved in the blood plasma have

as a rule high sedimentation constants and high molecular weights. The only exception is the blood pigment of the Chironomus larvae, which has a sedimentation constant almost identical with that of the cyclostomes and the capitellide worms.

Within a well-defined animal group all the species have, as a rule, the same sedimentation constant. All the polychaete worms and hirudineans with the respiratory protein dissolved in the blood have the constant 57.1×10^{-13}. Some of these proteins are red (erythrocruorin) others are green (chlorocruorin). The oligochaete worms have a constant 60.8×10^{-13} very close to that of the other worms. Some of the crustacean families show the sedimentation constant 16.9×10^{-13} (hemocyanin and erythrocruorin), others 23.4×10^{-13}, and one of them 34.1×10^{-13}. The xiphosurans and scorpions have the same constant, viz. 34.1×10^{-13}. All the gastropods except Planorbis and Arion have the constant 99.8×10^{-13}. It is obvious from these regularities that biological kinship is usually accompanied by identity in the sedimentation constant.

The determinations of molecular weight by means of sedimentation equilibrium measurements have given the surprising result that a system of simple multiples seems to obtain among the molecules of the blood pigments. The molecule of the gastropod hemocyanin has a weight of 5,100,000. The molecular weight of the erythrocruorin and chlorocruorin of the worms is about ½ of this figure. The erythrocruorin of Planorbis and the hemocyanin of the xiphosurans and scorpions is about ½ that of the worms. The hemocyanin molecule of sedimentation constant 23.4×10^{-13} is about ½ that of the Planorbis pigment, and the hemocyanin and erythrocruorin of constant 16.9×10^{-13} is about ½ that of the former.

This scheme of simple multiples for the molecular weights of the respiratory proteins is supported by some observations about the reversible dissociation of their molecules into simple submultiples at the borders of the pH stability regions. It is further strengthened by previous observations of simple relationships between the molecular weights of the proteins of lower weight.

The determinations of the isoelectric points of the respiratory proteins show that the blood pigments of the invertebrates are all much less alkalin than the hemoglobin of the vertebrates. It is of great interest to notice that the isoelectric point varies from species to species. Even very nearly related forms have different isoelectric points. For a genus containing several subgenera the isoelectric points lie closer together within a subgenus. The situation of the isoelectric point is therefore to a certain degree a measure of the kinship.

(This abstract is based on a lecture presented at the Marine Biological Laboratory on July 11.)

SOME EFFECTS OF BLUE-GREEN ALGAE ON LAKE FISH

(*Continued from Page 77*)

able pest in certain Iowa lakes is the blue-green alga *Aphanizomenon flos-aqua*. It grows in such abundance as to make some entire lakes or parts of lakes a thick, green "soup" at various times during the summer months. This plant has the characteristic habit of readily concentrating by wave and wind action into great thick mats or blankets, collecting along the shores and in shallow bays where the growth decays, thereby bringing about many objectionable conditions.

It was found that such superabundant growths of algae suddenly bring about the death of many thousands of fish in three ways.

First: The plants were so abundant that at night, when oxygen release by photosynthesis was not in progress, the oxygen demand for this great mass of organisms was so great that the dissolved oxygen content became lowered to a point that would not support fish life. In comparison with other aquatic plants, blue-green algae are, at best, poor oxygenators. Death of fish by this manner of suffocation was not found to be common.

Second: When algae grew in such abundance the unbalanced condition brought about their sudden death and disintegration. Their death and subsequent bacterial decay occurred in periods of high temperature when the oxygen supply was normally low due to the inability of warm water to hold oxygen. The bacterial decay apparently rapidly depleted the oxygen supply necessary for small animals which died in prodigious numbers. In turn their decay further depleted the oxygen supply to the point that fish were suffocated. Small lakes or bays in certain larger lakes were observed to have scarcely one living thing in them but bacteria. Even bottom organisms were killed. After such a crisis, dead fish whiten thousands of feet of shore line.

Third: It was noticed in areas where masses of algae were disintegrating, although not necessarily decomposing, that fish were dead or dying, apparently not from lack of oxygen. Dissolved oxygen readings showed sufficient oxygen to be present to support fish life (4.0 ppm).

Such observations naturally raised the question as to the cause of the distress and death of the fish and the idea of a possible poisoning occurred. To test for this a number of simple experiments were carried on in a stripping shed laboratory.

Large amounts of *Aphanizomenon* (20 to 50

gallons) were collected and allowed to decay in closed receptacles. In some experiments open receptacles were used. Ten different species of fish were seined from the lake and placed in a holding tank. After the algae were thoroughly decayed the vats were emptied into a large tank. The tank was aerated by means of oxygen of pipe drilled with fine holes being fitted to the end of the inlet hose. This dispersed the gas in the water and increased the solubility.

The fish were put into the tank with the decayed algae and their behavior observed. The D. O. at the time was 4.0 ppm. Intermittently oxygen was introduced and frequent D. O. readings were made to be sure that the supply was adequate for the fish.

At the end of one hour the sheepshead and perch used in the experiment were behaving abnormally. They dashed wildly about, driving into the sides of the tank and rolling on their sides. They showed no signs of being in distress for oxygen. There was no gulping at the surface nor did they gather about the oxygen inlet as they likely would have done had they been in need of oxygen.

Soon other kinds of fish showed signs of distress. The crappies, buffalo, perch, and sheepshead died at the end of 90 minutes with the D. O. at 4.6 ppm. At the end of 6 hours all fish of all species were dead with the D. O. at 4.2 ppm. Similar experiments showed the same effects, indicating that the fish had not died from lack of oxygen and strongly suggesting that they had been poisoned.

Decomposed algae were analyzed chemically in an attempt to determine whether or not poisons were present. Algae decomposed in closed containers and masses decomposed in open receptacles in the sun were used in the analyses. Simple tests showed an abundance of inflammable gas or gasses to be produced, at least when the plants decayed in closed receptacles. Since blue-green algae have an enormous protein content it was not surprising that large amounts of protein decomposition products were found. Among these was hydroxylamine. This is soluble in water and is very poisonous, at least for land animals and presumably for fish. While quantitative determinations were not successful it seems possible that sufficient amounts of hydroxylamine might be formed by decaying algae in restricted areas in the lake to bring about a poisoning of the fish. A search through the available literature has, to date, offered no information on the possible killing of fish by hydroxylamine or definite information concerning the poisoning of fish by blue-green algae at all.

Hydrogen sulphide was also found to be given off in large amounts by decaying blue-greens which also might have had detrimental effects.

While the experiments strongly suggest poisoning as the cause of death further observations are needed, particularly on the factors involved in natural conditions.

Although there is a strong feeling held by some that copper sulphate is objectionable as an algacide to inhibit such detrimental growths of the blue-greens, nevertheless it would seem to have its advantages. In this investigation fish were never found to be killed by copper sulphate nor were there significant detrimental effects on fish-food organisms. If some fish should be killed by improper use of the chemical, certainly many times more fish are killed directly and indirectly by the algae.

As far as is known there has been no work on the by-products of blue-green algal decomposition. The biological aspects and the possible commercial use of these products deserves study and consideration.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 5).

EXPERIMENTAL CYTOLOGY OF AMOEBA PROTEUS LEIDY

WM. L. DOYLE[1]

Research Assistant in Physiology, The Johns Hopkins University

The granular material in the cytoplasm of *Amoeba proteus* contains the following structures: (1) granules about .25 micron in diameter, called alpha granules by Mast ('26); (2) spheroidal vesicles about 1 micron in diameter, called beta granules by Mast ('26) and secretory granules by Metcalf ('10); (3) bodies with all essential characters of mitochondria; (4) truncated bipyramidal crystals enclosed in vacuoles; (These crystals often have small blebs attached to them.) (5) fat globules; (6) highly refractive globular bodies, called Glanzkörper by Greef ('74), spherical bodies by Mast ('26), secondary nuclei by Calkins ('05), and Monica Taylor ('24), Golgi bodies by Brown ('30), and nutritive spheres by Monica Taylor ('32).

By crushing the refractive bodies under the coverglass and by microchemical tests we have demonstrated that some of them contain a distinct fragile wall of carbohydrate material which surrounds a central plastic mass, and that the others are apparently homogeneous in structure. Mast

and Doyle ('32). Those with centers are scattered throughout the cytoplasm. In solubility and staining reactions they clearly resemble plastids. (Cf. Zirkle '29 and '33). These will be referred to as spherical bodies A. The homogeneous bodies (spherical bodies B) are found in the food vacuoles. They have the characteristics of the structure called vacuome by Volkonsky ('33). The solubilities and staining reactions of spherical bodies A and B and the blebs on the crystals show these elements to be similar.

In attempting to trace the transformation of food into cytoplasm by a study of the origin and function of these structures we made the following observations: If an amoeba is suspended in soluble starch and centrifuged, the fat globules aggregate at one end and the spherical bodies A at the other, and the remaining structures in fairly distinct regions between. By operating on chilled centrifuged amoebae some of the structures described can without serious injury be partially or entirely removed so as to ascertain their function.

Such operated specimens were either starved or fed on selected food organisms. The results obtained are presented in the following table:

Crystals	Sph. Bod. A	Mito.	Food	Result
Present	Present	Present	None	S. B. A. reduced in size.
"	"	"	Chilo.	S. B. A. increased in size. Crystals arise in food vacuoles.
"	"	"	Bacteria	S. B. A. increase in size. Crystals decrease in number followed by increased S. B. A.
"	Absent	"	Chilo.	No S. B. A. formed.
Absent and cut out as formed.	"	"	"	No S. B. A. formed.
Present	"	Present	"	S. B. A. formed.

S. B. A.=Spherical bodies A.
Mito. =Mitochondria.
Chilo. =Chilomonas paramecium.

The relation of mitochondria and crystals to spherical bodies A indicated in the table was confirmed by three other distinct methods. One of these consisted of direct microscopical observation of operated specimens containing so few spherical bodies A and crystals that each was individually recognizable. It was found by this method that if the specimen under observation contained a Chilomonas food vacuole 16 to 30 hours old, numerous mitochondria were in contact with the wall of the vacuole and that some were leaving and others arriving. It was also found that in the vacuole there was a spherical body B which was decreasing in size. It was further found that the mitochondria which leave the walls of the vacuole come in contact eventually with either a spherical body A or a crystal vacuole, that after a considerable number of mitochondria had been in contact with a spherical body A and the crystal vacuole, the former was increased in size, and a small bleb had appeared on the crystal, and that this bleb increased in size while the crystal decreased in size until the crystal disappeared and the bleb was indistinguishable from a spherical body A. It was found moreover that crystals arrive in the cytoplasm by subdivision of the food vacuole.

(This article is based on a seminar report presented at the Marine Biological Laboratory on July 18.)

[1] In collaboration with Prof. S. O. Mast.

THE BIOLOGICAL LABORATORY

COLD SPRING HARBOR

Dr. Reginald G. Harris
Director of the Biological Laboratory

The Biological Laboratory at Cold Spring Harbor commences, with this issue, the experiment of using a definite section of The Collecting Net. It is not always wise to announce the beginning of an experiment. Experiments, like demonstrations, have a surprising ability of "going wrong." The beginning of this particular experiment will be so obvious to all readers of The Collecting Net, however, that we are left no choice but to announce it, and, hence, it seems desirable to give some of the reasons why it is being undertaken.

Ever since the Biological Laboratory was transferred from the Brooklyn Institute of Arts and Sciences to the Long Island Biological Association, some ten years ago, we have been giving sustained thought to the rôle which the Laboratory should play in the progress of biology. We definitely took the point of view, stated in one of my "reports" published in The Collecting Net a few years ago, that, although useful ends could be accomplished by the development, at Cold Spring Harbor, of a small edition of the Marine Biological Laboratory of Woods Hole, still, a greater contribution to biology might be made by the development, at Cold Spring Harbor, of a laboratory employing somewhat different methods in the furtherance of the common cause. During the last several years certain steps which have thus far been taken, from time to time, to accomplish this, have been reported in The Collecting Net. This has been done with the hope of receiving constructive criticism, as well as of making a report to additional numbers of biologists, for whom all independent biological laboratories should exist and must function.

The latest distinctive move which we have made is concerned with the adoption, as part of our summer work, of a formal method of carrying into effect a portion of our policy of fostering a closer relationship between biology and the basic sciences, mathematics, physics and chemistry. This method involves the calling together of a group conference, each summer, in which representatives of the sciences mentioned will carry on research, confer upon, and discuss, some one phase of modern biological research. The mechanics of the method, as it is being used here, were described in some detail in the July 8th issue of The Collecting Net.

At that time it was stated that an important part of the plan included the making available of certain results of a given conference, to the profession at large, through the publication of a volume containing the lectures and symposia delivered at the conference, and such parts of the discussion as seemed significant or creative.

Since then, an offer to make use of a part of The Collecting Net, for the immediate publication of papers and discussions, has been accepted.

There are certain possible disadvantages connected with our acceptance of this offer. Outstanding among these is the possibility that the sale of Volume I of Cold Spring Harbor Summer Symposia in Quantitative Biology may be adversely affected by the availability of part of the papers in The Collecting Net.. If this develops to be the case the possibility of our meeting the cost of publication through the sale of Volume I, is, obviously, appreciably diminished. On the other hand, we hope that biologists in Woods Hole during the summer, and other readers of The Collecting Net, will find the papers delivered at conferences at Cold Spring Harbor, of more than enough value to counterbalance possible financial loss to the Biological Laboratory.

The Biological Laboratory exists for the advancement of biology. The new idea of extended formal conferences, as outlined, has been inaugurated for the advancement of biology. We welcome any practicable means of making the Biological Laboratory, and results of its work and conferences, more generally useful and available. At present The Collecting Net seems to furnish a valuable opportunity to do this, particularly in respect to workers at the Marine Biological Laboratory of Woods Hole.

At the same time we hope that more workers at Cold Spring Harbor will make use of The Collecting Net, and through its pages become better acquainted with research being conducted at Woods Hole. And finally that biologists, who do not make use of either station, will have available a source of information concerning work being conducted, and ideas being born, at both of these Laboratories.

These are some of the reasons why we are making the experiment of using a definite section of The Collecting Net.

THE THEORY OF THE DIFFUSE DOUBLE LAYER

DR. HANS MÜLLER
The Massachusetts Institute of Technology

It is a curious fact that the phenomenon which led to the discovery of electric charges, namely the production of electricity by friction, is even now one of the least investigated and least understood fields of physics. We know practically nothing about the exact mechanism producing frictional electricity. We know, however, that friction is not at all required to produce these charges. The mere contact of two materials gives rise to a potential difference. Friction does not create, but only increases, these potentials. Contact potentials exist on every boundary between two different phases, independent of whether one or both phases are electric conductors or insulators. The two phases need not even be chemically different, they may only differ in temperature or in cristallographic orientation.

According to the laws of electrostatics a potential drop in a surface is always connected with the existence of an electric double layer. A potential drop creates an electric field, and, according to Gauss's law, electric lines of force can only originate from positive electric charges, and they can only disappear at the seat of negative charges.

Hence in Fig. 1a we must have positive charges where the potential begins to drop, and negative charges where the potential gradient vanishes. Mathematically this relation between potential ϕ and charge density ρ is expressed by Poisson's equation

$$\frac{d^2\phi}{dx^2} = -\frac{4\pi\rho}{D} \qquad (1)$$

where x is the normal distance to the surface and D the dielectric constant. The body which is at the higher potential carries, therefore, along the boundary a layer of positive charges, and the other body carries a layer of negative charges, the charge density of both layers being equal. The ensemble of the two layers is called an electric double layer.

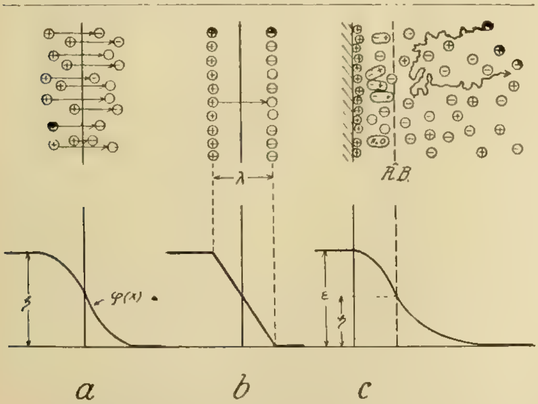

Fig. 1. Charge and Potential distribution in an electric double layer. a. General case. b. Helmholtz's double layer. c. Diffuse double layer. RB = Rigidity boundary.

The distance between the two layers is about the same as the distance in which the entire potential drop occurs. In general this distance will be very small, perhaps of the order of magnitude 1 to 100 $\mu\mu$. Due to this small thickness, it is practically impossible to observe the course of the ϕ (x) curve, and we must approach the problem from purely theoretical ground.

Helmholtz, who first introduced the electric double layer, assumed that the potential drop can be approximated by a straight line as in Fig. 1b, the entire drop occurring within a definite region of thickness λ. This assumption leads to the locating of each layer of charges within a geometrical surface. If the surface is plane the two layers form a parallel plate condensor, with a separation λ between the plates, and we have the well known equation for a plate condensor

$$\zeta = \frac{4\pi\sigma}{D} \cdot \lambda \qquad (2)$$

Where ζ is the total potential drop, σ the charge per cm.^{2} on either layer, and D the dielectric constant of the medium between the layers.

This equation of Helmholtz gives the important information, that the contact potential does not only depend on the charges σ accumulated on the surface, but also on the thickness λ of the double layer. But this theory does not give any information concerning the magnitude of λ. In all earlier investigations λ was, therefore, considered to be constant, and all changes of ζ were ascribed to changes of the charge σ.

Experimental and theoretical evidence points definitely to the fact that Helmholtz's picture of the double layer is too simple. Electric charges, whether they are electrons or ions, are subject to temperature motion. If, therefore, one of the bodies is a liquid or a gas, the charges can not be located in a geometrical plane. A rigid layer is only possible on the surface of a solid where the forces (adsorption, chemical binding, lattice forces, Van der Waal forces) are so strong that they reduce the temperature motion to a minimum. In a liquid or gas, however, the charges are free to move, and the electric layer will be diffuse.

The theory of the diffuse double layer was developed independently by Gouy [1] for the case

in which the charges are electrolytic ions, and by Mie [2] for the case in which the charges are electrons in gas or vacuum.

For colloidal and biological problems only the first case is of importance. Let us consider, (Fig. 1c), the boundary between a solid and an electrolyte. We assume the solid to be at the higher potential, its surface is, therefore, positively charged. These charges are most probably ions attached rigidly to the surface by "adsorption" forces. Passing from the solid into the liquid we will pass a definite "rigidity boundary." On the solid side of this boundary the temperature motion is so small that it can be neglected. This boundary need not necessarily coincide with the surface of the solid or its adsorption range, it may lie outside the solid phase. We shall see that in the immediate neighborhood of the surface the electrostatic forces are so large that there the temperature influence is quite negligible. Around the solid there is, therefore, a film of liquid which is rigidly held by the solid. Our rigidity boundary is identical with Freundlich's [3] "Abreissschicht." The theory of the diffuse double layer is a theory of the electrokinetic potential ζ only and does not include the phase potential ϵ.

The total charge per $cm.^2$ on the rigid side of the boundary is denoted by σ. This charge is not necessarily adsorbed, but part of the charge may be located between the adsorption range and the rigidity boundary.

On the liquid side of the rigidity boundary the electrolytic ions can move freely. According to the law of equipartition of energy each ion has, on the average, an energy of translation equal to $^3/_2\ kT = 6\ 10^{-14}$ erg. Near the surface the negative ions will be attracted by the positive surface charge σ. The energy of this attraction is $z\ e\ \zeta$ where z is the valency and e the electronic charge. If the energy of attraction were very large compared with the temperature energy, the negative ions would fall into the surface. But the electrokinetic potentials are usually smaller than 100 Millivolts, hence

$$e\,\zeta \cong 4.77\ 10^{-10} \cdot \frac{0.1}{300} = 16\ 10^{-14}\ \text{erg}$$

is usually of the same order of magnitude as $^3/_2\ kT$.

If, therefore, a negative ion happens to come near the surface it has still enough energy to escape again from under the influence of the attraction force. The attraction will, however, force it to linger relatively longer near the surface than anywhere else. Consequently we will find, in the time average, more negative ions near the surface than far away from it. The negative ions are in a situation quite analogous to the molecules of air in the atmosphere. In the atmosphere the gas molecules are attracted to the surface of the earth by gravity, but the gravity is not strong enough to force all the molecules to the ground. The consequence of the attraction is, that we have more air molecules, that is, a higher pressure at sea level than at higher altitudes.

The positive ions, on the other hand, are repelled by the positively charged surface. While some positive ions will always penetrate near the surface, their number will, in the time average, be small, compared to the number far away from the surface.

Far away from the surface the charge/$cm.^3$ of all negative ions is equal to the charge of all positive ions. Since near the surface we have more negative and less positive ions than in the solution, we have there a surplus of negative charges. These charges form the negative layer of the electric double layer. This layer is not located in a definite plane, but is distributed. We have an "ionic atmosphere" or a "diffuse double layer." The irregularity of the Brownian motion makes it impossible to calculate at any instant the exact distribution of the ions, but we are able to give the time average of the distribution of the charges, and hence calculate the time average of the potential. The instantaneous potential difference ζ will fluctuate very little and very rapidly about this average value, and what we measure is this time average.

Mathematically the problem is best formulated by using the procedure given by Mie and Debye [4]. We use the analogy with the pressure distribution in the atmosphere. In the atmosphere we have the barometer equation

$$\log \frac{p}{p_o} = \log \frac{n}{n_o} = -\frac{M\,g\,h}{R\,T} = -\frac{m\,g\,h}{k\,T}$$

Where p is the pressure, n the number of molecules at the height h, and p_o, n_o their values at the elevation $h = o$. M is the molecular weight, g the gravitation constant, R the gas constant and T the absolute temperature. $m = M/N$, ($N =$ Avogadro's number) is the mass of one molecule and $k = R/N = 1.37\ 10^{-16}$ is Boltzmann's constant. In this equation gh is the potential of the gravitational field, and mgh the potential energy of the molecule. In the case of ions we have the same equation, except that the gravitational potential is replaced by the electric potential ϕ, and instead of the mass we have to introduce the charge of the

ion. For any arbitrary kind of ions of valency z_i we have, therefore,

$$\log \frac{n_i}{n_i^o} = - \frac{z_i e \phi}{k T}$$

or (3)

$$n_i = n_i^o e^{-\frac{z_i e \phi}{kT}}$$

This law can also be derived in a more general way from Maxwell-Boltzmann's principle. n_i is the number per cm.^{3} of ions of valency z_i (z_i with the sign corresponding to the charge of the ion) in a point where the average electric potential ϕ exists. n_i^o is their number where $\phi = O$, that means in the electrolyte far removed from the surface. n_i^o is determined by the molar concentration c_i according to

$$n_i^o = c_i \cdot 6.06 \; 10^{20}$$

The charge density ρ is then

$$\rho = \Sigma n_i e z_i = \Sigma n_i^o e z_i e^{-\frac{z_i e \phi}{k T}} \qquad (4)$$

Introducing this in Poisson's equation (1) gives the fundamental differential equation of the potential distribution in the diffuse double layer

$$\frac{d^2 \phi}{d x^2} = - \frac{4 \pi}{D} \Sigma n_i^o e z_i e^{-\frac{z_i e \phi}{k T}} \qquad (5)$$

If we replace

$$\frac{d^2 \phi}{d x^2}$$

by the Laplacian $\Delta \phi$ this differential equation holds for any kind of surface.

Multiplying (5) by $d \phi$ and integrating from $x = \infty$ to an arbitrary value x gives,

$$x = \infty, \; \phi = O \text{ and } \frac{d \phi}{d x} = O$$

$$\tfrac{1}{2} \left(\frac{d \phi}{d x}\right)^2 = + \frac{4 \pi k T}{D} \Sigma n_i^o \left(e^{-\frac{z_i e \phi}{k T}} - 1\right) \qquad (6)$$

At the rigidity boundary $\phi = \zeta$ and according to Gauss's law

$$\frac{d \phi}{d x} = - \frac{4 \pi \sigma}{D}$$

hence (7)

$$\sigma = \pm \sqrt{\frac{D k T}{2 \pi} \Sigma n_i^o \left(e^{-\frac{z_i e \phi}{k T}} - 1\right)}$$

where σ has the same sign as ζ. This equation for the plane double layer permits one to calculate the surface charge σ from the measured concentration c_i and the electrokinetic potential ζ.

There are two important cases for which this formula can be simplified.

Case 1.

The potential ζ is so small that

$$z_i \frac{e \zeta}{k T} < 1$$

That is the case if $z_i \zeta$ is smaller than 25 Millivolt. We can then develop

$$e^{-\frac{z_i e \zeta}{k T}} = 1 - z_i \frac{e \zeta}{k T} + \tfrac{1}{2} \left(z_i \frac{e \zeta}{k T}\right)^2 - \cdots$$

Introducing this in (7) and taking into account that $\Sigma n_i^o z_i = O$ (which expresses the fact that the total charge in the electrolyte is zero) gives

$$\zeta = \frac{4 \pi \sigma}{D} \cdot \frac{1}{\kappa} = \frac{4 \pi \sigma}{D} \lambda \qquad (8)$$

where

$$\kappa^2 = \frac{1}{\lambda^2} = \frac{4 \pi e^2}{D k T} \Sigma n_i^o z_i^2 \qquad (9)$$

We find again Helmholtz's equation. In first approximation a diffuse double layer is equivalent to a Helmholtz double layer, but the thickness of the double layer is now determined by (9). Introducing the value for water at room temperature one gets

$$\lambda = \frac{4.32 \; 10^{-5} \text{ cm}}{\sqrt{z_i^2 \gamma_i}}$$

where γ_i is the concentration expressed in micromols per liter.

We observe that, for concentrations smaller than 1/10 molar, the thickness of the double layer is considerably larger than the diameter of an ion.

This fact justifies the statistical method employed. For higher concentrations, however, the calculations are questionable.

Introducing the series for

$$e^{z_i \frac{\epsilon \phi}{k T}}$$

in equation (6) we get by a simple integration* the potential distributions in the double layer

$$\phi(x) = \zeta e^{-\kappa x} \qquad (10)$$

The potential ϕ decreases exponentially with the distance x from the rigidity boundary. At a distance $x = \lambda$ the potential has the value $\phi = \zeta/2.718$ and at the distance $x = 2\lambda$, $\phi = \zeta/(2.718)^2$ etc.

According to equation (9) the thickness of the double layer decreases with increasing concentration, the decrease being the faster the higher the valency of the ions. Since the observed ζ potentials have these same properties, we believe that the variations of the ζ potentials are primarily due to the change of the double layer, and to a much less extent due to changes of the adsorbed charges.

Introducing (8) in (3) we can finally determine the distribution of the ions near the surface.

$$n_i = n_i^o e^{-\frac{z_i e}{k T} \zeta e^{-\kappa x}}$$

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* Introducing

$$e^{-z_i \frac{e \phi}{k T}} - 1 = - z_i \frac{e\phi}{k T} + \tfrac{1}{2} \left(z_i \frac{e \phi}{k T}\right)^2$$

in (6) gives

$$\tfrac{1}{2}\left(\frac{d\phi}{dx}\right)^2 = -\frac{4 \pi k T}{D} \left\{ -\frac{e\phi}{kT} \Sigma n_i^o z_i + \tfrac{1}{2}\left(\frac{e\phi}{kT}\right)^2 \Sigma n_i^o z_i^2 \right\}$$

and since the first sum vanishes

$$\frac{d\phi}{dx} = - \sqrt{\frac{4\pi e^2}{D k T} \Sigma n_i^o z_i^2} \cdot \phi = -\kappa \phi$$

which leads directly to (8), (9) and (10).

This double exponential gives a very rapid increase in the number of negative ions and a fast decrease in the number of positive ions near the positively charged surface. The first approximation is, however, only valid if $z\,\zeta < 25$ Millivolt, and this condition is seldom satisfied. It is therefore, important to study the more general case where an accurate solution can be given.

Case 2.

If the electrolyte consists of only two kinds of ions of the same valency we have

$$n_1^o = n_2^o = n$$

$$z_1 = - z_2 = z$$

and equation (7) reduces to

$$\sigma = 2\kappa \frac{D k T}{4 \pi e z} \sinh \frac{z e}{2 k T} \zeta$$

Using the abbreviation

$$\frac{z e}{k T} \zeta = \Psi$$

we can write this

$$\sigma = \frac{D}{4\pi} \zeta \kappa \cdot \frac{\sinh \Psi/_2}{\Psi/_2}$$

Comparison with (8) shows that the first approximation leads always to too small values of σ. In order to get the correct value of the charge the results of the first approximation have to be multiplied by a correction factor which depends on the value of Ψ. Some values of this correction factor are given in Table 1.

TABLE 1.

Ψ	$\frac{\sinh \Psi/_2}{\Psi/_2}$	Ψ	$\frac{\sinh \Psi/_2}{\Psi/_2}$
0.6	1.015	7	4.73
0.8	1.027	8	6.83
1.0	1.042	9	10.0
2.0	1.175	10	14.84
3	1.42	12	33.62
4	1.81	14	78.33
5	2.42	16	186.31
6	3.34	18	450.17
		20	1101.32

Contrary to the general belief the correction is quite appreciable as soon as ζ is larger than 50 Millivolt for monovalent, and more than 25 Milli-

volt for bivalent, ions. The integration of equation (6) is, in this case, also possible and gives*

$$\phi = \frac{4\,k\,T}{z\,e}\ \text{tangh}^{-1}\, A\, e^{-\kappa x} \qquad (11)$$

Where tangh^{-1} is the inverse function of the hyperbolic tangent and A is an integration constant determined by the ζ potential

$$A = \text{tangh}\ \frac{z\,e}{4\,k\,T}\ \zeta$$

For a discussion of (11) it is better to substitute $A = e^{+\kappa x_0}$ and use again

$$\Psi\ (x) = z\ \frac{e\,\phi}{k\,T}$$

then

$$\Psi = 4\ \text{tangh}^{-1}\ e^{-\kappa\ (x - x_0)} \qquad (11')$$

This function is plotted in Fig. 2 and compared with the first approximation (8) which in the same notation is

$$\Psi = e^{-\kappa\ (x - x_0)} \qquad (8')$$

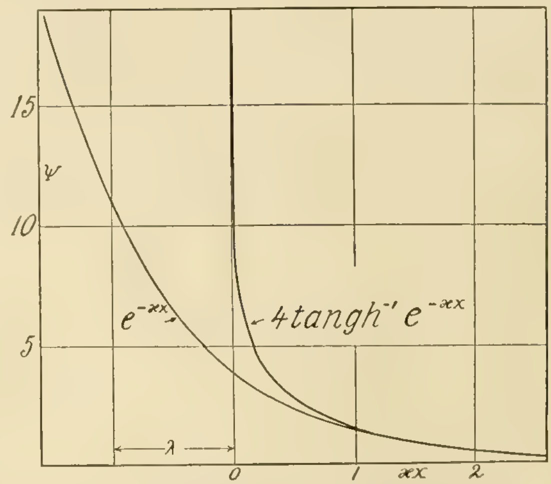

Fig. 2. Comparison of potential curves in a plane diffuse double layer using the first approximation and the accurate solution for a Z - Z valent electrolyte.

We observe that the approximation is only good as long as $\Psi < 2$. For large values of Ψ the potential drop is very much steeper than given by the first approximation. For instance the drop from $\Psi = 20$ to $\Psi = 10$ occurs in the distance $^1/_{100}\ \lambda$ and the drop from $\Psi = 40$ to $\Psi = 20$ in less than $^1/_{10000}\ \lambda$. But usually $\lambda < 10^{-5}$ cm. is of the size of the ionic radii. Consequently the theory of the diffuse double layer must break down for high potentials. We can interpret this fact in a more elucidating way. Let us assume that the theory of the diffuse double layer could be applied for high potentials Ψ and that it did hold for the ϵ-potentials. We use the reasonable values $\epsilon = \frac{1}{2}$ Volt, $\Psi = 20$, $\lambda = 10^{-6}$ cm. Since Ψ drops to half its value within a distance $^1/_{100}\ \lambda = 10^{-8}$ cm we get near the surface of the adsorbed charges an electric field strength of

$$\frac{\frac{1}{4}}{10^{-8}} = 25 \text{ Million Volt/cm.}$$

This result justifies our statement that the field near the surface of the particle is tremendous. It

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*With the assumption

$$n_1^0 = n_2^0 = n,\ z_1 = -z_2 = z$$

equation (2) is

$$\frac{1}{2}\left(\frac{d\,\phi}{d\,x}\right)^2 = \frac{4\,\pi\,k\,T\,n}{D}\left(e^{+z\frac{e\,\phi}{k\,T}} - 2 + e^{-z\frac{e\,\phi}{k\,T}}\right)$$

But

$$e^{y} - 2 + e^{-y} = (e^{+y/2} - e^{-y/2})^2 = 4\ \sinh^2 y/_2$$

Hence

$$\frac{d\,\phi}{d\,x} = -\,2\,\kappa\ \frac{k\,T}{e\,z}\ \sinh\ \frac{z\,e}{2\,k\,T}\ \phi$$

From which follows the result (10).

Introducing

$$\Psi = \frac{z\,e}{k\,T}\ \phi \qquad \text{gives}$$

$$\frac{d\,\Psi}{d\,x} = -\,2\kappa\ \sinh\ \Psi/_2 = -\,4\kappa\ \sinh\ \Psi/_4\ \cosh\ \Psi/_4$$

Whence

$$\frac{d\ (\Psi/_4)}{\sinh \Psi/_4 \cdot \cosh \Psi/_4} = -\,\kappa\ d\ x$$

Or

$$\frac{d\ (\text{tangh}\ \Psi/_4)}{\text{tangh}\ \Psi/_4} = -\,\kappa\ d\ x$$

Hence by integration

$$\log\ \text{tangh}\ \Psi/_4 = -\,\kappa\ x + \text{const.}$$

Which leads directly to the above result.

is so large that it annihilates any temperature influence. We have no knowledge of the behavior of matter in such strong fields, but theoretical speculations lead us to believe that these fields not only will deform the ions, but will also act on the water molecules. The field will line up the electric dipoles of the water and hold the molecules in a definite orientation. Connected with this effect is a large hydrostatic pressure which will be discussed in another paper. In the region of these strong fields the liquid will, therefore, adhere to the solid and the rigidity boundary is moved towards the liquid. The ζ potential is, therefore, not so large as the total potential drop ϵ. The largest values ζ can assume is about 100 Millivolts, corresponding to maximum field strength of 100000 Volts/cm.

If the surface is not plane, but spherical, as in the case of colloidal particles, or cylindrical, as in capillaries, the differential equation (5) has only been solved for the case

$$\frac{z\,e}{k\,T}\,\zeta < 1.$$

The result is in both cases the same: The diffuse double layer is equivalent to a Helmholtz double layer of thickness $\lambda = 1/\kappa$. For the sphere the two layers form a spherical condensor, and hence

$$\zeta = \frac{4\,\pi\,\sigma}{D}\;\frac{\lambda}{1 + \lambda/r}$$

and for the cylinder

$$\zeta = \frac{4\,\pi\,\sigma}{D}\,r\log\,(1 + \lambda/r)$$

where r is the radius of the sphere or the cylinder respectively.

Where $\Psi > 1$ the problem of the spherical double layer has been investigated by La Mer, Gronwall and Sandved[5] and by the writer.[6] The first three authors gave a series development of Ψ, while I developed a graphical method. It is found that the first approximation is not sufficient in quite the same way as for the plane double layer.

Using this graphical method I was able to show, that measurements of the ζ potential of an $As_2\,S_3$ colloid, carried out by Freundlich and Zeh, could be satisfactorily explained by assuming that the charge σ remained constant. The theory does not only give the correct dependence of the concentration, but also of the valency of the ions. According to the approximate theory a four valent ion, for instance, should have twice as strong an influence as 4 monovalent ions. Experimentally one finds, however, that the influence of a 4 valent ion is about 2000 times stronger than a monovalent ion. The higher approximation is able to explain this difference, and gives results in agreement with the observation. Finally, the theory is also in good agreement with the observed temperature dependence of the ζ potential. Burton found that the cataphoretic mobility of colloids varies with temperature as the inverse of the viscosity. This indicates that the product ζ D must be constant. Assuming σ to be constant we have

$$\zeta\,D = 4\,\pi\,\sigma\sqrt{\frac{1}{D\,T}\;\frac{4\,\pi\,e^2}{K}\;\Sigma\,n_i^0\,z_i^{\,2}}$$

and we find indeed that $\sqrt{DT}$ varies in water between 0 and 100° by less than the experimental error of Burton's measurements.

Since the theory of the diffuse double layer is based on generally accepted principles, and explains the outstanding properties of the electrokinetic potentials, we should accept it as correct. The theory, however, does not explain individual properties of the ζ (c) curves, like maxima and isoelectric points. These properties must be explained by changes of the adsorped charges.

DISCUSSION

Dr. Fricke: Regarding your discussion of Freundlich and Zeh's measurements, does your assumption, that the charge is independent of the electrolyte concentration, signify a belief that the charge is chiefly due to the adsorption of ions other than those in the solution, as, for instance, H ions.

Dr. Müller: The assumption of a constant charge is not necessarily correct. Freundlich and Zeh's data agree with this assumption only, if the particle radius is 15.8$\mu\mu$. If the particles were smaller we must assume an increase, if they were larger we must assume a decrease of the charge, with increasing concentration. But since a radius of 15.8$\mu\mu$ leads to constant charges, independent of the valency of the ions, it is most probable that this assumption is here correct. The nature of the adsorbed ions is probably determined by the preparation of the colloid, they are not ions of the added electrolyte.

Dr. Abramson: In general all surfaces can be divided into two groups. In the first group are the inert surfaces such as quartz and paraffin oil. The charge of these surfaces is particularly sensitive to changes in the salt concentration, and, as Dr. Müller and I have shown, the charge increases with increasing salt concentration reaching a limiting value. The charge-concentration curve is very much like the Langmuir adsorption isotherm. In the second group, the charge seems to be mainly determined by particular ions. That is,

a protein surface has its charge determined by the activity of the hydrogen ions. Of course salts also shift the isoelectric point of proteins, and, consequently, the salts may also modify the charge of proteins. But in spite of the fact that there is no sharp line between these two groups, the description of the change in concentration curves, just given, is most useful.

Dr. Fricke: What is the order of magnitude of the thickness of the adsorbed water layer?

Dr. Müller: This layer is probably monomolecular, its thickness is of molecular dimensions, as calculations of Gyemant show.

Dr. Fricke: What is the present state of the idea that the electrostatic image force is responsible for adsorption?

Dr. Müller: For the theory of the ζ potential, the image force does not have to be considered, since Poisson's equation is used, and hence all electrostatic forces are taken into account. For the adsorption process, the image force is important. It is the basis of the adsorption theory of Jaquard and Hückel.

Dr. Cole: Since the image force depends on the dielectric constant of the solid, and decreases rapidly with the distance, it should be considered as a molecular force of the type of Van der Waal forces.

Dr. Curtis: What value did you use for the dielectric constant of water, in view of the large fieldstrength at the double layer?

Dr. Müller: If we neglect the hydration of the ions in the double layer, the use of the ordinary dielectric constant is probably justified in the entire range of the diffuse double layer, because the fieldstrength does not reach values above 100000 Volt/cm. Within the rigidity boundary, however, the dielectric constant must be smaller, since we will get dielectric saturation. The correct way to treat this problem is by using a molecular, rather than the classical, theory of dielectrics. This was done by Gyemant.

Dr. Blinks: Is the rigidity boundary a definite surface, or is it also diffuse?

Dr. Müller: Since the electric fieldstrength diminishes very rapidly with the distance from the surface, the rigidity boundary is probably very sharp. Assuming a diffuse boundary, would create great theoretical difficulties. It is, however, feasible that the distance between solid and rigidity boundary changes with the electrolyte concentration.

Dr. Cole: The existence of a rigid layer around the particle, and the large hydrostatic pressure in the rigidity boundary, would justify the use of the ordinary viscosity in the formula for the cataphoretic migration speed. Some experiments by Bond, on the apparent resistance of spheres of liquids, give the result that, above a certain radius, not only the viscosity of the exterior, but also the viscosity of the interior, plays a role. In the case of small particles the existence of the rigid layer probably eliminates the influence of the viscosity of the interior.

Dr. Abramson: Dr. Müller's rigidity boundary is probably only acceptable for solid surfaces. I do not think that large oil droplets, such as those investigated by Mooney, can be treated in such a fashion. Internal rearrangements of the oil drop itself, incidental to the bodily movements of the drop, can certainly modify the chemical constitution of the surface.

Dr. Mudd: Does the theory of the diffuse double layer apply also to instances where the rigid part of the double layer is composed of ionogenic matter, such as protein? That is, are the surfaces of the material itself ionized?

Dr. Abramson: I would like to point out to Dr. Mudd that this theory actually has its best support in experiments with proteins. Using this theory 60 per cent. of the theoretical absolute value of the charge of protein molecules was calculated from electrophoresis measurements.

Dr. Cole: Is there any justification for assuming the ϵ potential to be ½ Volt or larger?

Dr. Müller: If the measurements of ϵ are correlated with the electrocapillary curve, values of this magnitude are found. It is, however, correct that the use of the capillary electrometer is not free from objections.

Dr. Fricke: I should like to direct your attention to electric polarization as a means of studying the diffuse double layer. I refer to polarization of the irreversible type, as observed at a metal electrode in an inactive salt solution. As you know, the polarization is equivalent to a capacity, the polarization capacity, in series with a resistance, the polarization resistance. The polarization capacity may be considered to be a measure of the reciprocal of the thickness of the double layer which is built up by the electric current. Measurements of the polarization capacity for different frequencies of the electric current give the thickness of the double layer as a function of time, and show a high value initially, which decreases within a fraction of a sec-

ond to a constant value. Presumably, the decrease is due to adsorption, and it is interesting to note that the decrease over a considerable range of time takes place according to t^{-x}, where x is often about .30. This equation is similar to one used by Freundlich to account for adsorption as a function of time. These remarks must not be taken to mean that a complete theory of this type of polarization is available, but their purpose is to direct your attention to an experimental method which appears promising for the study of the diffuse double layer.

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THE THEORY OF ELECTROPHORETIC MIGRATION

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A rigorous test of the theory of the diffuse double layer requires the knowledge of the electrokinetic potential ζ as a function of the electrolyte concentration. Using this theory, it is then possible to calculate the charge of the colloidal particles. The ζ potential can be measured with the help of any one of the electrokinetic phenomena. Frequently, however, the method of the cataphoretic migration is the only experimental method possible. This is the case in respect to many biological systems, such as bacteria or blood corpuscles.

Electrophoretic or cataphoretic migration is the phenomenon of the migration of colloidal particles in an electric field. All the theories developed so far give the result, that the velocity V of the particle is given by

$$V = \frac{X D \zeta}{K \pi \eta} \qquad (1)$$

where X is the strength of the applied field, D the dielectric constant of the liquid, and η its viscosity. According to this formula a particle moves towards the positive pole, if its potential, and hence its charge, is negative. Its velocity is proportional to the ζ potential. The variation of the velocity gives directly the change of ζ. For a quantitative investigation, however, it is of the greatest importance to find the absolute values of ζ. This requires the knowledge of the numerical constant K introduced in equation (1). Unfortunately, the various theories lead to different values of this constant. I propose, therefore, to compare, here, the various theories, their assumptions, consequences and limitations. The purpose of this study is to make a decision concerning the numerical constant K.

If a spherical particle of radius R and carrying a positive charge Q is placed in a uniform electric field of strength X it will come under the influence of a force QX. This force will produce an accelerated motion of the particle in the direction of the field. If the particle is suspended in a liquid, the motion will produce frictional forces proportional to the instantaneous velocity of the particle. The friction will decrease the acceleration, and finally cancel it entirely, when the frictional force is equal to the accelerating force. It can be shown that, for colloidal particles, this state is reached in an immeasurably short time. We observe, always, the unaccelerated state in which the particle has a steady velocity V.

The frictional force on a spherical particle is, according to Stockes,

$$F_o = 6 \pi \eta R V$$

Hence we have for the steady state

$$QX = 6 \pi \eta R V$$

and

$$V = \frac{X}{6 \pi \eta} \frac{Q}{R} \qquad (2)$$

The potential ζ_o of a sphere in a medium with the dielectric constant D is

$$\zeta_o = \frac{Q}{D R} \qquad (3)$$

and the above equation (2) can, therefore, also be written

$$V = \frac{X D \zeta_o}{6 \pi \eta} \qquad (4)$$

This equation is correct for a sphere in a perfectly insolating medium. For a colloidal particle this equation is, however, not valid. Neither Stockes' law, nor equation (3) for the potential, is applicable. This is due to the fact that a colloidal particle is surrounded by an electric double layer. As discussed in the preceding article, the positive charges of the particle attract the negative ions of the electrolyte, and repel the positive ions. The

liquid in the intermediate neighborhood of the surface is thus negatively charged. The electric field does not only act on the positive charge of the particle, but it acts also on the negatively charged liquid. The liquid will move in the direction opposite to the motion of the particle. The velocity of the particle, relative to the immediately surrounding liquid, is, therefore, increased. The friction force depends, evidently, on this relative velocity. Stockes' force F_o must, therefore, be replaced by a larger frictional force F.

On the other hand, the electric double layer changes also the potential of the particle. In the first approximation we have

$$\zeta = \frac{Q}{DR} \frac{1}{1 + {}^{R}/\lambda} = \zeta_o \frac{1}{1 + {}^{R}/\lambda} \qquad (5)$$

where λ is the thickness of the double layer.

The increased friction force will decrease the velocity V of the particle. Equation (2) can not hold. But according to equation (5) the potential also is decreased. There exists, therefore, the possibility that these two effects cancel each other in such a way that the final result (4) is, nevertheless, correct. This is certainly the case if the thickness of the double layer is very large compared with the radius of the particle. In this case the negative charges are far away from the particle, and the velocity of the particle, relative to its immediate surrounding, is the same as in Stockes' law. According to (5) the difference between ζ and ζ_o vanishes for $\lambda >> R$. Equation (4), giving K=6 is, therefore, correct, in the limiting case where the thickness of the double layer is much larger than the radius of the particle. Any theory of the cataphoretic migration speed must, for this limiting case, give K=6.

It is possible, but not necessarily correct, that equation (4) can hold for any arbitrary thickness of the double layer. This would be the case, if the electrophoretic motion of the liquid increases the friction force according to the law

$$F = F_o \ (1 + {}^{R}/\lambda) \qquad (6)$$

We would have then

$$QX = 6 \pi \eta \ RV \ (1 + {}^{R}/\lambda)$$

and using (5) we would get again

$$V = \frac{XD\zeta}{6 \pi \eta}$$

There are, however, no reasons which would justify equation (6). We can not even expect that the new friction force can be represented as the sum of Stockes' force F_o and an additional electrophoretic force. This is due to the circumstance that the equations of hydrodynamics of a viscous fluid are not linear, and the principle of superposition is not valid. To give a theory of the cataphoretic migration speed, it is necessary to find a solution of an entirely new hydrodynamical problem different from Stockes' law. One has to find a solution of the differential equation

$$\eta \left(\frac{\delta^2 v_x}{\delta x^2} + \frac{\delta^2 v_x}{\delta y^2} + \frac{\delta^2 v_x}{\delta z^2} \right) + \frac{\delta p}{\delta x} + F_x = O$$

and two analogous equations for v_y, v_z satisfying the condition of incompressibility

$$\frac{\delta v_x}{\delta \chi} + \frac{\delta v_y}{\delta y} + \frac{\delta v_z}{\delta z} = 0$$

And the boundary conditions, that at infinity v_x=v, v_y=o, v_z=o, and at the surface of the rigidity boundary $v_x = v_y = v_z = o$. In these equations v_x is the velocity of the liquid in the x direction, p the hydrostatic pressure, and F the force exerted on the liquid by the electric field.

The first solution of the problem was given by von Smoluchowski[1]) in 1903. In this derivation he makes the following assumptions.

1. The presence of the particle produces a distortion of the electric field in such a way, that the electric current passes tangentially along the surface of the sphere.

2. The double layer is so thin, that the electric field can be considered to be parallel to the double layer in the entire range of the latter. While Smoluchowski considered a Helmholtz double layer, this is not important. His derivation holds for any double layer satisfying the above condition. This was shown later by Gyemant.[2]

3. The electric field does not deform the double layer.

Smoluchowski finds the solution

$$V = \frac{XD\zeta}{4 \pi \eta}$$

He even could prove that this answer is quite independent of the shape of the surface. Spherical, ellipsoidal, cylindrical or arbitrary shaped particles should have the same migration speed.

Smoluchowski's formula is different from Stockes' formula by the factor 4π instead of 6π. It does not, therefore, satisfy the important condition of the limiting case. But this is not required, since assumption 2 limits the formula to hold only for very thin double layers. It can not

be extrapolated to thick double layers. Smoluchowski realized this deficiency of his equation, but he could not explain it.

It was, therefore, of great value that Debye and Hückel[3] attacked the problem again in 1924. They solved anew the system of differential equations, but without making use of assumption 2. They proved that, however thick the double layer might be, an equation of the type

$$V = \frac{D X \zeta}{K \pi \eta}$$

is always valid, but they found that the constant K should vary with the shape of the particle. For the sphere, Hückel calculated the value K=6.

Hückel's equation satisfies the limiting case, but his result is in definite disagreement with Smoluchowski's. It is not correct to explain this discrepancy, as Freundlich does, by saying the factor 4 is valid for a rigid, and 6 is valid for a diffuse, double layer. Hückel's calculation should hold for any kind of double layer, and so should Smoluchowski's result, provided the diffuse double layer is thin enough.

This discrepancy gave rise to a long controversy. Eliminating the possibility that either calculation contains a mathematical error—which is not the case—the question can only be settled in two ways.

One way is the experimental method. This method, of course, can not tell which factor is the correct one, but it can definitely settle the question whether or not the factor changes its value with the form of the particle. The first comparison of cataphoretic migration speeds of differently shaped particles was performed by Van der Grinten.[4] He verified Debye's contention. But Abramson[5] could show that the interpretation of his results is not correct, and Abramson gave definite experimental proof that Smoluchowski's, and not Debye's, conclusion is correct. The migration speed does not vary with the shape of the surface. While this result indicates that the factor 4 might be correct, it does not exactly disprove Hückel's calculation, and it does not explain why Smoluchowski's result does not satisfy the limiting case.

The second way to solve the dilemma is to compare the assumptions made in the two derivations. We have mentioned, already, that the change of assumption 2 can not be the reason for the discrepancy. Assumption 3 is also accepted in Debye's and Hückel's paper. However, assumption 1 is different in the two derivations, and this accounts for the different results. It is rather curious that this difference was only discovered in 1931 by D. C. Henry.[6] Debye and Hückel assume, that the particle does not distort the electric field. If the particle is of a non-conductive material, a distortion certainly does exist. The reason why Debye and Hückel neglected this, is probably to be found in the fact, that they were primarily interested in the electrophoretic force acting on an ion. An ion is so small that it does not distort the outer field. The entire double layer around an ion is in a uniform field, and Hückel's result is unquestionably correct for this case. If the particle is, however, of colloidal size, then the question arises: is the distortion of the field large enough to change the result? The answer can evidently be given as follows: if the thickness of the double layer is so large that the greatest part of the double layer is so far away from the particle that the distortion of the field is negligible there, then Hückel's result is still valid. Now, the larger the particle, the farther the distortion of the field will extend in the liquid. Consequently, Hückel's result can be accepted only if the thickness, λ, of the double layer is large, compared with the radius R of the particle. If, however, the ratio λ/R is small, then the distortion of the field is important. D. C. Henry has studied this case, and again solved the differential equation, using a general double layer not subject to restriction 2, but taking into account assumption 1. He verifies Smoluchowski's result if

$$\lambda/R \leqq 1$$

Henry finds, as we should expect, that Hückel's result is valid if λ/R is large. He calculates that λ must be 600 times larger than the radius of the particle, in order that the factor 6 is justified. For values of λ/R between 1 and 600 the numerical constant increases from 4 to 6, the variation being calculated by Henry. Henry's paper solves the dilemma between Hückel's and Smoluchowski's result. His work shows, that both results are correct in two different limiting cases, and his answer evidently satisfies the condition of the limiting case.

I wish to point out, however, that Henry's work should still be improved in two directions. In calculating the distortion of the electric field around the particle, Henry uses the classical method. He considers the liquid as a continuum with a uniform conductivity. This is evidently not quite correct. The concentration of the electrolytic ions is different in the double layer from that in the electrolyte. Hence the conductivity will change near the surface of the particle. The experiments on surface conductivity give definite evidence of this change. The distortion of the electric field can, therefore, be considerably dif-

ferent from the one assumed in Henry's paper. This circumstance does not destroy the validity of the two limiting laws, but it will give a different change of the numerical factor with λ/R. Even less satisfactory is Henry's consideration of the case where the particle is an electric conductor. While Henry realizes, that this case is probably not in accord with his theory, I would like to point out that he neglects the fact, that real electric charges of opposite sign are accumulated on the boundary where the current enters and leaves the particle. These charges would produce an asymmetric double layer.

A second improvement is necessary, due to the fact that Henry accepts the third assumption, namely, that the outer field will not change the charge distribution in the double layer. This assumption is probably justified for very thin double layers. If the thickness of the double layer is small, the electric field in the double layer is large, (over 1000 Volt/cm.), and the small outer field, of a fraction of a Volt/cm., can have no appreciable effect. If, however, the thickness of the double layer is large, a large region of the ionic atmosphere is under a weak field, and the outer field can pull the ions of different signs to opposite sides of the particle. The double layer is then asymmetric. Debye and Hückel have calculated this effect for ions. The asymmetry produces an additional force on the particle, decreasing its velocity. Using Debye's and Hückel's result it can easily be shown that this effect is also of importance for colloidal particles. Mooney [7] reports a new formula for the electrophoretic velocity, which takes into account this effect, but since he does not give its derivation, it is not possible to discuss his method. Since the asymmetry reduces the velocity, particularly where Hückel's solution is otherwise valid, we must expect that the numerical factor can reach values even larger than 6.

There is, finally, an additional difficulty, which will modify the electrophoretic migration speed. In the formulation of the hydrodynamical problem, it was supposed that the electric field acts on the liquid carrying the charges of the double layer. Actually the field acts on the ions. The question arises, therefore: is the entire force exerted on the ions, transferred to the liquid, or is only a certain part of this force active. At present this question can not be answered. We have, however, good reason to assume that, in a very thin double layer, the entire force is transferred from the ions to the liquid.

Summarizing, we can, therefore, state:

The classical formula of Smoluchowski is correct, if the thickness of the double layer λ, and the ratio $\lambda/_R$, are small. Its exact range of validity can not be given at present, and it is doubtful whether it holds for conductive particles. Outside of this validity range, the numerical factor is larger than 4 and may reach values larger than 6. Within its validity range, Smoluchowski's formula is probably applicable to any shape of surface, but outside this range the numerical factor will depend on the shape of the particle.

Discussion

Dr. Abramson: You brought out that the numerical factor may be as high as 10, if the theory is corrected. Using Henry's approximation and Debye's theory to calculate the charge, I get instead of a charge of 5 electrons on the egg albumen only 3 at p H. 4.0. If the factor 10 were used perfect agreement would be obtained between the charges calculated from electrokinetic, and thermodynamic, measurements

Dr. Fricke: Would you like to discuss the theoretical case produced when the charge on a rather large particle, such as a protein molecule, consists of a few electrons only?

Dr. Müller: The theory considers only the average value of the ζ potential. It is conceivable, that the potential varies along the surface, particularly if the charge consists of only a few ions on the surface of a large particle. But, due to the temperature motion, the particle will rotate, and only an average value can be observed.

Dr. Mudd: It would be of interest to calculate the case, where different parts of the particle have a different ζ potential. Rabbit spermatozoa. for instance, dead or alive, migrate in a cataphoresis cell tail foremost. When the direction of the current is reversed, they are slowly reoriented until they again move tail foremost. This must be due to the tails having higher ζ potentials than the heads.

Dr. Abramson: I have confirmed Dr. Mudd's explanation by studying amino-acid needles having small oil droplets attached to one end. Similar orientation phenomena were observed.

Dr. Cole: There is one thing that is extremely interesting in this apparent contradiction between the case of the thin, and the case of the thick, double layer. The difference lies in the force on the double layer. If the double layer is thin, it is in the field existing near the particle. If it is thick, it is in the uniform field. If one has a nonconductive sphere in a continuous medium, one finds that the maximum field strength, which occurs on the equator normal to the field, is exactly 50% greater than the field at some dis-

tance from the surface. This difference of 50% accounts for the difference between 4π and 6π.

Dr. Abramson: Do you mind discussing the fact that the orientation of cylindrical particles does not influence their electric mobilities?

Dr. Müller: If the long axis of the cylindrical particle is parallel to the electric field, it offers a small hydrodynamic resistance. If the axis forms a right angle with the field this resistance is much larger. On the other hand, the electric field will be less distorted in the first case than in the second case. The smaller the hydrodynamic resistance, the larger the mobility, and, as we have seen, the smaller the distortion of the electric field, the larger the mobility of the particle. In Smoluchowski's case of a very thin double layer, these two effects just cancel each other, since an orientation with a small hydrodynamic resistance produces also a small distortion of the field.

Dr. Abramson: In other words, if the factor 4π is valid, you would expect that the electric mobility should be independent of the orientation.

Dr. Müller: Yes. If, however, the double layer is thick, the factor will probably depend on the orientation. For a very large thickness of the double layer, the mobility will be the larger, the smaller the hydrodynamic resistance.

Dr. Cole: In the matter of independence on size and shape, when one has a thin double layer, I should like to mention that, one of the simple ways of mapping hydrodynamic lines of flow consists in building up a model of an insulator, and then sending the electric current in at the place where the current of fluid would come in, and take it out where the fluid would come out. The hydrodynamic lines of flow coincide, then, with the flow of the electric current. The same differential equations apply to the viscous flow, that apply to the electric flow. The hydrodynamic resistance is, therefore, calculated with the help of the same picture as that used for the calculation of the electric stream lines. Consequently, one has no effect of the shape of the particle. As a corollary to that, there is no orienting effect on the particle, as long as the surface charge is uniformly distributed.

Dr. Müller: This analogy is correct only outside of the double layer, where the electrophoretic force can be neglected.

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THE REACTION OF KIDNEY TUBULES TO NEUTRAL RED AND TO PHENOL RED

DR. ROBERT CHAMBERS

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From observations on fragments of chick mesonephros in tissue culture, it has been shown definitely that phenol red, the salt of a highly dissociated sulphonated acid dye, is picked up by the cells of the proximal tubules, passed into the lumen of the tubules, and accumulated against a considerable concentration and pressure gradient. This action takes place in spite of varying conditions of acidity within and without the tubule as long as the metabolic activity of the renal cells is maintained. Respiratory poisons, such as KCN, CO, H_2S and cold, upset the oneway passage of phenol red through the cells. This effect can be reversed when inhibiting conditions are removed.

With neutral red, a basic dye salt, the situation is quite different. It is a vital stain and behaves as such only under certain pH conditions, irrespective of the metabolic activity of the renal cells. It penetrates and stains indiscriminately all the cells of the tubule, proximal, distal and of the collecting duct, and then only when the acidity of the environment is less than the acidity of the interior of the cells. It will not pass into the lumen of the tubule unless the acidity of the fluid in the lumen is more than that of the surrounding cells, a condition which obtains in tissue culture only in the case of the distal tubules.

In brief the passage of phenol red into the tubule depends upon the metobolic activity of the renal cells while that of neutral red is controlled by differences in acidity irrespective of whether the cells are narcotised or not.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 14.)

THE EFFECT OF RESPIRATORY POISONS AND METHYLENE BLUE ON CLEAVAGE OF CERTAIN EGGS

By M. M. BROOKS
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In these experiments, cyanide and carbon monoxide, which are well-known from Warburg's work to poison the respiratory enzyme, were used. A brief account of the rôle of methylene blue in counteracting the effects of these poisons follows.

That methylene blue is an antagonist for cyanide was first shown by Thünberg in 1917, using succinic acid and a dehydrogenase. He found that the oxidation of succinic acid which was stopped by cyanide, was resumed when methylene blue was also present. In 1924 St. Gyorgy, using Thünberg's idea, showed that the O consumption of crushed muscle tissue could be restored after it was stopped by cyanide when methylene blue was added. In 1926 Sahlin used this idea in white mice, injecting them with a lethal dose of cyanide and then causing them to recover when methylene blue was injected. This is the first time that whole animals had been used in this connection. In 1928 Barron and Harrop showed in some beautiful experiments using living cells in vitro, that the oxygen consumption of these cells could be restored by methylene blue after it had been stopped by cyanide. In 1931, Eddy again showed this antagonism in dogs. In 1932 I showed the same effect in rats, and then suggested from all this evidence that methylene blue should be used in human cases. This suggestion was relayed by Dr. Hanzlik to Dr. Geiger in San Francisco five months later when an emergency case was brought in.

In the case of carbon monoxide, omitting the early contributions which showed that CO stopped O consumption, Warburg showed in 1930 that methylene blue in certain concentrations antagonizes the effects of CO in living cells *in vitro*. On the basis of these results, I injected rats with methylene blue after they had been poisoned with CO; immediate recovery followed. This was published in 1932. These were the first experiments using whole animals in this connection. I then suggested that this antagonism could be applied to human cases in monoxide poisoning, and asked Dr. Geiger to try it out in an emergency case which promptly appeared and very promptly recovered on injection of this dye.

Following these announcements, various writers have attacked the theory suggested in explanation of these experiments, the experiments themselves, and the clinical evidence.

As to the theory, following Dr. Barron's interpretation that the action of methylene blue on cyanide is that of a catalyst, and later, Warburg, who in 1930 discussed this action at some length, I advanced as a working hypothesis, the idea that also in my experiments, methylene blue acted as a catalyst.

However, Hug in Oct. 1932, and Wendel in April 1933, working independently, both came to the conclusion that the action of methylene blue in whole animals when injected into the blood stream in cyanide poisoning, is not one of a catalyst, but rather is due to the formation of methemoglobin which promptly unites with cyanide, taking it out of the blood stream. These authors also found that the nitrites had the same property, a finding which has been supported by recent work of Clowes and Chen.

Since methemoglobin has no such affinity for CO as it has for cyanide, Henderson objected that there was no theoretical ground for using methylene blue in monoxide cases, and also on the basis that it required a large concentration of CO to inhibit oxidation in tissues as compared with the small concentrations fatal to whole animals. However, the fact still remains that methylene blue is an antagonist for CO in whole animals and therefore a theory must be found to fit the facts.

In order to see if any light could be thrown on this problem, I studied the cleavage of sea urchin eggs and star-fish eggs, this being an activity of the cell closely related to or dependent upon cell oxidations. Furthermore, since these cells have no hemoglobin, the cyanmethemoglobin reaction postulated by Hug and Wendel need not be considered. These eggs were subjected to KCN or CO or N, and also placed in these solutions plus various concentrations of methylene blue, fifteen minutes after fertilization. Although these experiments are only preliminary, it was found that cleavage is retarded by both CO and Cyanide. The effect with cyanide has been previously shown. Methylene blue acts as an antagonist for CO in the cleavage of *Arbacia* eggs if the proper concentration of dye is used; it increases the rate of cleavage in low concentrations and inhibits it in higher concentrations; the antagonism in *Asterias* is not proven in these experiments; the dye antagonizes the effects of cyanide on cleavage in proper concentrations.

(This article is based on a seminar report presented at the Marine Biological Laboratory on July 14th.)

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

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PUBLICATION

The question as to whether the printing of an article in The Collecting Net constitutes publication is a pertinent one. Technically, it does; practically perhaps it does not. Typewritten or monographed copies of an article can be copyrighted and distributed. In the technical sense, then, even this method is construed as publication. The answer to the question depends upon the definition of the word "publication." In the sense that it was used by Dr. Conklin the other day it does not constitute publication. He defined publication in a "recognized scientific magazine" as the accepted method of obtaining priority. We do not consider The Collecting Net a "recognized scientific magazine." We do not want it to become one.

The Collecting Net is glad to have the privilege of printing preliminary reports of research work, but that is not its only purpose. There are people who believe that we print a great deal of extraneous material which they feel it would be wiser to omit. One or two trustees of the Marine Biological Laboratory have chosen not to submit their seminar reports to The Collecting Net because they do not think it is dignified to have them printed in a magazine which includes local news, expresses opinions, and is generally rather informal. Fortunately they are in the minority.

That we do not aspire to mold The Collecting Net into an accepted medium for obtaining priority by the publication of research reports may seem puzzling to many people. We have long considered the possibility of attempting to become an accredited scientific magazine and in fact, only recently made the tentative proposal to the Marine Biological Laboratory that the magazine become affiliated with it in at least a semi-official way, and that it be edited under the direction of an editorial board appointed by the Laboratory. To all intents and purposes it would have then become an adjunct to The Biological Bulletin. Under these conditions it would perhaps not differ enough from existing publications to warrant its continuation.

We have recently sought the advice of many people concerning the most useful way of conducting The Collecting Net, and as a result have decided to make no radical change in our policies. It will remain independent for the present. It will continue to foster informality in its contents and to seek material of interest to the biologist even though it may not always be biological. It will intentionally avoid becoming a strictly scientific publication by including material which no recognized scientific publication would consider within its province. By fostering this spirit of informality authors will have freedom in the expression of opinion; they can make statements and review fields of work without appending long bibliographies. Writing an article for The Collecting Net will not be quite as much of a chore as laboring over one for a more serious publication. It will be a little more like delivering an extemporaneous speech and one can feel free to express an idea or opinion even though he realizes there is a possibility that it may be necessary to refute it six months later.

The Collecting Net is a product of the summer. It is read during the warm months when many biologists tend to relax somewhat from keeping abreast of the scientific literature in their fields. This is another reason why we do not want The Collecting Net to become too serious. It may be long before we obtain the kind of contributions that we want, but we will work toward that end.

Suggestions, verbally and in writing, are sought. Only with the help of everyone working in the field of biology can The Collecting Net hope to reach its maximum usefulness.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the hole turns to run from Buzzards Bay to Vineyard Sound:

July 22	4:56	5:03
July 23	5:32	5:41
July 24	6:12	6:23
July 25	6:52	7:05
July 26	7:31	7:49
July 27	8:13	8:35
July 28	8:57	9:25
July 29	9:44	10:21
July 30	10:36	11:18
July 31	11:34	

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

ITEMS OF INTEREST

Dr. Robert Chambers will leave Woods Hole on Sunday and plans to return the following Thursday. On Wednesday he will take part in the symposium at Cold Spring Harbor on "Oxidation-reduction Potentials," giving a paper on "Intra-cellular oxidation-reduction potentials." Earlier on the same day Dr. L. Michaelis will discuss "The Reversible Two-step Oxidation." These papers constitute a small part of an extensive program at the Biological Laboratory on "The Potential Difference at Interfaces and Its Bearing Upon Biological Phenomena."

Dr. Alfred C. Redfield is making plans for an extensive cruise of the *Atlantis*, the purpose of which will this time be an investigation of the formation of bottomwater in the North Atlantic The deep layers will be followed northward until the region where they reach the surface is approached This cruise is scheduled to begin on August 12 and it will extend over a period of at least a month.

Several investigators from Woods Hole are planning to attend the Third International Congress for Experimental Cytology, which will convene in Cambridge, England, from August 21-26. Dr. L. Michaelis is chairman of the section on the "Electrophysiology of the Cell." He will read a paper entitled, "The Reduction Intensity of the Living Cell." A paper entitled, "The Relation Between Ions and Potential Differences Across Plasma Membranes," will be given during this session by Dr. S. C. Brooks.

The Tenth Annual Meeting of the Long Island Biological Association will be held at Blackford Hall, Cold Spring Harbor, on Tuesday, July 25, 1933, at 6:30 P. M. *Agenda*: Ratification of acts by the Board of Directors and Executive Committe; report of the Laboratory Director; report of the Treasurer; election of seven directors of the class of 1937; such other business as may be brought before the meeting.

The Michigan Academy of Sciences has undertaken to publish a 200-page treatise on the "Fresh Water Algae of Newfoundland" by Dr. Wm. R. Taylor who is director of the course in marine botany at the Marine Biological Laboratory. Thirty plates accompany his manuscript.

Dr. B. H. Grave left Woods Hole Monday afternoon for his home at Greencastle, Ind., where he is professor of zoology at DePauw University.

Dr. Brooks is planning to leave for England on July 30 on the *Britannic*. The meeting of the Physiological Society at Plymouth will be his first objective, and from there he will go on to the Congress at Cambridge. Mrs. Brooks is returning to the University of California to continue her investigations there.

Mr. Robert A. Nesbit, assistant biologist in charge of starfish investigations at the Cambridge station of the U. S. Bureau of Fisheries, visited the Woods Hole station for three days last week, returning to Cambridge on Thursday evening.

Miss Margaret Grierson, a member of last year's invertebrate course, was married to Dr. E. C. Cole, professor of biology at Williams College and in charge of the invertebrate course here, at Maplewood, N. J., home of the bride, on July 20th. Mrs. Cole is Associate in Zoology at the University of California at Los Angeles.

Mrs. Marie Laug has returned to Woods Hole from the Women's Hospital in Boston where she underwent an operation for appendicitis. Mrs. Laug is the wife of Dr. E. P. Laug, instructor of physiology at the University of Pennsylvania.

Dr. I. J. Kliger, Professor of Public Health at the Hebrew University in Jerusalem, is visiting Woods Hole this week with his wife and son, David, preparatory to leaving for Palestine. He has been spending the summer in Nantucket, having completed his work here in the interest of the University.

Dr. Fred W. Stewart of New York, who has been spending a month's vacation in Woods Hole, will leave Sunday to resume his duties at Memorial Hospital.

Miss Edwina Morgulis has returned to Woods Hole from Paris. Since her graduation from Radcliffe she has been studying French literature at the Sorbonne. She is the daughter of Professor and Mrs. Sergius Morgulis.

Some members of the Woods Hole younger set have embarked on a project known as The Summer Club. The membership includes Molly Rugg, Constance Heilbrunn, Frank and Martha Lillie, Margaret and Samuel Morris Jr. Mrs. L. V. Heilbrunn is sponsor of the club and Mrs. Samuel Morris, librarian. Plans for the summer include picnics and the building of a shack on which construction has already begun.

FERTILIZATION MEMBRANES OF CENTRIFUGED ASTERIAS EGGS

Donald P. Costello
Instructor in Zoology, University of Pennsylvania

The pulling apart of the eggs of marine invertebrates by means of a strong centrifugal force was early observed by Lyon (1907) for Arbacia, by Lillie (1909) for Chaetopterus (also Wilson 1929, 1930), and by Morgan (1910) for Cerebratulus. More recently E. N. Harvey (1931) has made use of this property to compute the tension at the surface of marine eggs, and E. B. Harvey (1932) has studied the development of the resulting cell fragments of the Arbacia egg.

Under ordinary conditions the fullgrown starfish egg remains immature, with its germinal vesicle intact, until it is removed from the body cavity of the animal into sea-water. About ten minutes later the nuclear membrane begins to disappear, and in about sixty minutes the first polar body is separated. Accompanying the breakdown of the germinal vesicle, as Lyon (1907) has shown, there is a recrease in the viscosity of the protoplasm so that it is possible to stratify the egg by centrifuging.

The experiments to be described are concerned only with the effects of centrifuging during the early maturation period, that is, between the breakdown of the germinal vesicle and the separation of the first polar body. Eggs were centrifuged in sea-water with an isotonic sucrose layer at the bottom of the tube, with the tube temperature at 20 to 25 degrees Centigrade in an electric centrifuge giving a force of about 6000 times gravity.

After the breakdown of the germinal vesicle, the egg is distinctly stratified and slightly elongated by twelve minutes of centrifuging. A few minutes later, the same amount of centrifuging results in the pulling apart of the egg inside of the jelly hull. Two fragments are produced, a centripetal light fragment containing the oil, hyaline zone, and some yolk spheres, and a smaller centrifugal fragment containing the remainder of the yolk.

These fragmented eggs, contained in the jelly hull, were fertilized immediately upon being removed from the centrifuge tubes. Within sixty seconds after inseminating, a fertilization membrane can be seen to separate from the heavy fragment. A partial membrane, or none at all, separates from the lower pole of the light fragment. Even in those eggs in which the fragments are not in contact, both fragments are usually activated, presumably by different sperm, and subsequently cleave. During the late morula stage, the effects of the presence and absence of a complete fertilization membrane is particularly striking. The heavy fragment produces a spherical morula, held together by a fertilization membrane. The light fragment produces a pear-shaped mass of cells which tends to bud off the blastomeres at the centripetal pole into the surrounding medium.

If the ovoid stratified egg, centrifuged immediately upon the breakdown of the germinal vesicle, is inseminated, the fertilization membrane separates in the normal manner from the heavy pole of the egg. Passing toward the light pole, the fertilization membrane is thinner and closer to the egg, and at the light pole, continuous with the egg surface. This is not merely an eccentric membrane, as the difference in membrane thickness demonstrates. During subsequent cleavage, a thin membrane may separate from the blastomeres at the light pole, but this membrane does not resemble, either in thickness or in proximity to the blastomeres, the fertilization membrane of the heavy pole.

The evidence is believed to demonstrate that at the appropriate stage of maturation, the substances responsible for membrane separation can be displaced into the heavy half of the egg by centrifugal force.

(This article is based on a seminar report presented at the Marine Biological Laboratory on July 14.)

IONIC CHANGES DURING THE DEVELOPMENT OF FISH EGGS

Dr. Laurence Irving and Jeanne F. Manery
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During the development of eggs of *Fundulus heteroclitus* the carbon dioxide content of the eggs increases. The increase in carbon dioxide is greater than could be accounted for by changes in carbon dioxide tension and consequently represents the development of an alkali reserve, which in turn indicates a gain in buffering power, as yolk material is synthesized into the embryo. In view of the significance of buffering power as a limitation upon the extent of vertebrate metabolism, particularly of the anaerobic type, it is worth while to see if the same change occurs during the development of other eggs.

The eggs of the speckled trout are more easily

examined in this respect because during fifty days they develop in fresh water. During this time, the carbon dioxide content of the eggs increases from about 1.3 to 5.2 cc. per 100 grams. The construction of carbon dioxide dissociation curves showed that the increase is connected with a true increase in carbon dioxide capacity. Normally the carbon dioxide tension in the eggs is constant at between 2 and 4 mm., so that the bicarbonate component is the one which gains. The proportion of bicarbonate increases from 2/3 to 9/10. This change represents an alteration in the average pH of the egg components from about 6.6 to 7.5.

A change in pH implies simultaneous alterations in other ionic components, which can be partially predicted if the ionic concentration of the egg remains constant, and from numerous observations in the literature this assumption is correct. As bicarbonate ions are gained, then other anions must be lost. It is natural to expect that loss of chloride ions would compensate for the bicarbonate increase.

Series of analyses of chloride contents of the eggs showed that the chloride concentration steadily diminishes. The chloride loss, however, is about six times the gain in bicarbonate, so that some other anions must replace the chloride. In view of the change toward alkalinity, the other anions must be those of weak acids. These might be supplied by the conversion of non-ionized phosphate compounds to ones which are ionized, a process occurring in the development of the hen's egg. Since the probable phosphate changes are inadequate, it is considered that with increasing alkalinity the dissociation of anions by proteins may supply a sufficient amount.

These alterations in the ionic components of fish eggs cannot be described in terms of the well known chloride shift of red blood corpuscles. The forces involved in the transfer of ions are, however, common and seem in the eggs to be furnished by metabolic changes of growth.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 14.)

COMPARATIVE STUDIES ON THE OSMIOPHILIC AND NEUTRAL-RED-STAINABLE INCLUSIONS OF THE GENUS VORTICELLA

HAROLD E. FINLEY
Associate in Biology, West Virginia State College

This report is concerned with the morphology and physiological significance of osmiophilic, argentophile and neutral-red-stainable inclusions in *Vorticella convallaria, V. microstoma, and V. campanula.*

In vital staining, dilutions of 1:20 were found to be satisfactory. The dyes were made up in stock solutions of 1% absolute alcohol and diluted with the same solvent. Vorticellae were studied in culture medium placed on slides previously filmed with the dye solutions.

The Da Fano method of silver impregnation, Mann-Kopsch (Weigl) and Kolatchev methods of osmic impregnation were used to prepare whole mounts and sectioned material.

In each species studied, globular cytoplasmic inclusions were stained vitally with neutral red. Certain of these inclusions measured 0.5 to 1 micron in diameter and they seemed to follow the cytoplasmic streaming. After staining with a mixture of neutral red and Janus green B, (equal parts of a 1:20 solution) the rod-shaped mitochondria could be distinguished from inclusions stained red.

Material prepared according to osmic and silver methods revealed definitely blackened globules measuring 0.5 to 1 micron in diameter. After these methods food vacuoles, nucleus and contractile vacuole were occasionally blackened.

Discrete globular cytoplasmic inclusions, demonstrable by recognized Golgi techniques and also stainable with neutral red have been interpreted by some investigators as "Protozoan Golgi material" and by others as the "vacuome."

It was pointed out that neutral red is not a specific stain for the vacuome in Protozoa. In Vorticella it consistently stained food vacuoles and other cell structures as well. Therefore, one should be positively conservative in regard to the identification of the vacuome after the use of neutral red. Other sources of errors included observations regarding the morphological peculiarities of the animals under consideration.

In view of the fact that at the present time we have no definite methods for determining the physiological significance of the vacuome, *in the Protozoa,* I must remain non-committal on that point. However, this investigation does not support the view that the vacuome is associated with food vacuoles in any way.

This point was emphasized: If it can be demonstrated that discrete osmiophilic to argentophile globules are universally present in Protozoa, and that they are of physiological significance, then their homologues in the metazoa will be of secondary importance.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 18.)

HEREDITY AND ENVIRONMENT*

Dr. Edwin G. Conklin, *Emeritus Professor of Biology, Princeton University*

When I was asked to speak to you this evening I agreed without any large amount of urging because I am very glad to see any church take up work of the sort that is implied in an open forum for discussion, where thoughts of people can be brought out and where questions can be asked. Indeed I have often thought, since I have been a teacher and lecturer for very many years, that it would be highly desirable if public speakers, whether in church or on a platform or in a classroom, were to be subjected to a grilling questioning after they finished speaking.

I have been announced to speak upon a rather hackneyed subject but one which is of the greatest importance to all of us, namely, which is the more important in the development of the individual, heredity or environment.

There are no two persons in this audience who look alike to me—among all the people of Falmouth I should not find two persons who were identically alike. There are, of course, so-called "identical twins" but even these show slight differences. Recent studies have shown that such twins differ in minor details although they have identical heredity, since they come from a single egg. In the whole world there are estimated to be about one billion eight hundred million persons and it is safe to say that no two are identical. How is it possible then to have such great differences in human beings who are still human? In the first place, all human beings, except for "identical" twins, have a different inheritance. The fact is that the units of heredity, which are now called genes, get so sorted that in every generation one-half of them that are carried by the father are thrown away, and one-half of those carried by the mother are thrown away. The remaining two halves unite to give rise to a new human being, but the units which are so united are never exactly the same. There is only one chance in several billions that two children of the same parents will have exactly the same genes. Nevertheless children of the same family have many genes in common and this accounts for certain family resemblances and traits. Besides physical resemblances there are also social and mental traits that are inherited, such as disposition, degree of intelligence, type of personality.

We must, however, also consider environment which plays an important part in the development of personality. A person may come from a family in which insanity is hereditary. This does not mean necessarily that he will become insane, but that he will be likely to do so under certain adverse conditions which a person with a different inheritance might be able to undergo unscathed. It is the same way with tuberculosis; the disease itself is infectious, but a person who has inherited slight resistance to tuberculosis would very probably develop it, *if* infected, while someone with a stronger resistance would remain immune under the same conditions. Whether one is calm or excitable, conservative or radical, law-abiding or criminal, may have their beginnings in inheritance.

So that we are not all equal by any means in the way in which we have been endowed by heredity. When Thomas Jefferson wrote in the Declaration of Independence, "we hold these truths to be self-evident, that all men are created equal . . . ," he did not mean that all men were equal as to color, or wisdom or ability; he merely referred to the fact that in a popular government, such as was being established in this country, all men were equal before the law. And yet men are not all equal before the law in responsibility. Children and insane persons are excepted, and even in the same individual, responsibility and capacity to resist temptation varies at different times. Responsibility rises and falls during the course of a day and lapses almost completely in sleep. What do we mean by responsibility? We mean the ability to respond to a situation in a reasonable, purposeful manner; the capacity, for example, to respond in a lawful manner rather than unlawfully.

Some people maintain that heredity has made us what we are entirely, that we have no choice in doing what we do. They quote the Scriptural text, "Which of you by taking thought can add a cubit to his stature?" A cubit is about two feet and that is quite a bit to add to one's stature, except by means of stilts; but we can, by taking thought, add or subtract two or three pounds of weight. A leopard cannot change its spots nor an Ethiopian his skin, but I have seen white people become very nearly the color of an Ethiopian on the beaches in the summer!

We are not absolutely free—our heredity, our background, our early experiences all have an influence over which we have no control. But we do have a large amount of control over our behavior through the formation of habits. Education itself is very largely the establishment of good habits, "conditioned reflexes" the physiologists call them, and a habit is something that has been learned or acquired. Good habits carry one along without very much effort, but habits are things which we are capable of modifying. We

* **Transcribed from shorthand notes of an extemporanious talk which Professor Conklin gave last Sunday evening at the Methodist Episcopal Church in Falmouth.**

do thus have a certain amount of power in shaping our own careers; we are not entirely bound by heredity nor by early environment, which we cannot change.

You see therefore that both heredity and environment are involved in the making of an individual. Heredity determines the *possibilities;* the realization of these possibilities has got to come about through their development under environmental conditions. Every one of us could have been a different person from what he is; given the same heredity each of us might have turned out something different and possibly better or worse. I think it was John Wesley who, on seeing a drunkard in the street, remarked, "There, but for the grace of God, goes John Wesley." This realization of what we might have been gives us a sense of sympathy and understanding. As the French proverb puts it, "To know all is to pardon all." If one "knew" more one could have more sympathy with offenders. They may not always need sympathy, but punishment should never be retributive, it should be made to fit not the crime but the criminal. Punishment should be inflicted for the training or reformation of the criminal or for the protection of society and not for any other reason. So I say, we should have in mind the fact that any one of us might have been different, might have been much better or much worse. Whittier wrote: "Of all sad words of tongue or pen, the saddest are these, 'It might have been'." I believe that even sadder than the words "It might have been" are these, "I might have been." Heredity has been kind to most of us, the possibilities within us are great but they rarely come to full fruition.

I used to talk more enthusiastically of eugenics than I do now. The world is moving too fast to wait for the slow workings of eugenics; education is a much more rapid process in enabling human beings to realize their best possibilities. Heredity is difficult to control. Only when you choose a mate with wisdom and forsight can this be controlled. There is no other way by which bad heredity can be made good. But we can take the heredity that is given us and develop the better possibilities, suppressing the worse ones, and come to a better type of human personality.

Question: What about Dr. Watson and the "School of Behaviorists"?

Dr. Conklin: Dr. Watson is reported to have said that if you were to give him one hundred babies (no one, of course, would be likely to do it) he would agree to make out of any of them doctors, lawyers, writers, or anything that was desired. It is interesting fact that the less people know about babies the more sure they are of what they can do with them. When they have babies of their own they discover that babies have tendencies of their own. This claim of Dr. Watson's is no more justified than an assertion that all babies are born of one color or one size. It might be possible to take a baby with the inherited qualities of a poet and bring him up as a lawyer, but you could be fairly sure that he would not be as successful or as happy as if he had followed his inherited bent. On the other hand, children who come of parents who cannot give them the proper opportunities are not able to develop their hereditary possibilities, but this does not change their fundamental endowments. Intelligence should be distinguished from education and knowledge; intelligence is the capacity to know, knowledge is the thing known. The *capacity* to know is inherited but not knowledge. Some people are born with one talent, others with ten; but none will develop unless used.

Question: Is man more than simply an organism? Is he free?

Dr. Conklin: Man is more intelligent and free than any other organism. Intelligence is the ability to regulate behavior in the light of experience, the ability to profit by past experience. Any individual, human being or animal, that can consciously profit by past experience is to that extent intelligent. A horse that learns to open a gate is intelligent with respect to that one point. Any being that can thus profit by experience, that can learn to avoid mistakes and to repeat successes, is intelligent in that respect. Freedom is the ability to control behavior by means of intelligence; the more intelligent we are the freer we are. A large amount of behavior is purely mechanistic and cannot be self controlled. There are many other processes which human beings can control, such as the formation of habits, by means of which they can free themselves from the mechanistic compulsion to which other creatures must submit. Wherever there is intelligence there comes this possibility of directing behavior, halting before acting, and profiting by past experience of others as well as of one's self. I do not want to be more free than that—free to discriminate and choose between given alternatives in the light of past experience. This is a mechanistic type of freedom for whatever the ultimate nature of discrimination and choice may be they are causal phenomena. Science deals with phenomena that can be reduced to mechanistic principles and laws. Human behavior conforms to certain laws. There is therefore no such thing as absolute freedom. One can only choose between alternatives that are open to him. Human beings sometimes choose the worse alternative, because they are not aware of it, because they think that it may be changed later to the better, or perhaps in a spirit of bravado, just to see how much they can "get away with."

CONSTITUTION AND BY-LAWS OF THE M. B. L. CLUB

Constitution

Article I.

The name of the club shall be the Marine Biological Laboratory Club.

Article II.

The object of the Club is to promote social intercourse among the scientific workers of the Woods Hole community and their friends.

*Article III.**

The membership of the Club consists of two classes, active and associate. The scientific workers of the Woods Hole community and members of their families, eighteen years of age or over, are eligible to active membership, and become members on payment of the annual dues. Other persons who are eighteen years of age or over may be elected to associate membership as provided in the By-Laws.

Only active members in good standing have the right to vote. With the exception of voting, the associate members have all the rights and privileges accorded to the active members.

The annual dues for active and for associate membership are one dollar* for the fiscal year beginning with the annual meeting.

The families of members are entitled to the use of the Club without further payment of dues, provided that this privilege is not extended to less than eighteen years of age.

Article IV.

The officers of the Club shall be the President, the Vice-president, and the Secretary-Treasurer*. Only those active members who are in full standing and who are also members of the Corporation shall be eligible to these offices.

The officers shall be elected at the annual meeting; they shall be subject to recall as provided in Article VII of the Constitution. Vacancies occurring at other times shall be filled as provided in the By-Laws.

Article V.

The annual meeting shall be held each year one week after the official opening of the courses, at which meeting the election of officers, the presentation of official and standing committee reports, and other stated business shall be transacted.

Article VI.

There shall be an Executive Committee of which the officers of the Club shall be *ex officio* members. Other members of this Committee shall be appointed by the President to serve for one year, subject to discharge by him at any time.

Article VII.

Special meetings of the Club for any purpose except that of amending the Constitution may be called at any time by any officer of the Club; they may also be called by petition by at least 15 active members who are in full standing; the purpose and date of such meeting shall be stated in a notice which shall be posted in a public place in each of the Laboratory buildings, and in the Club-House, at least five days before the proposed date of such meeting.

Article VIII.

Amendments to this Constitution can be made by a two-thirds vote of those active members who are present at any meeting, *provided* that at least 30 such members are present, or provided that one-third of the total active membership below 90 is present, and *provided* that the proposed Amendment and the petition for the meeting to consider such Amendment or Amendments, and the date of such meeting, shall have been signed by at least 10 voting members and posted in a public place in each of the Laboratory buildings and in the Club-House at least 10 days before the date of the meeting.

By-Laws

1.* The Executive Committee shall consist of nine members, and shall include the officers of the Club and the Chairmen of the House and Social Committees.

The duties of this Committee shall be to attend to such general affairs of the Club as the running of the Club-House, and the appointment and discharge of a House Committee, a Social Committee, a Membership Committee, and such special Committees as it may deem necessary.

The Committee shall also have the power to fill vacancies in the offices of the Club occurring at other times than at the election at the annual meeting.

The Committee shall further have power to elect those persons to associate membership who do not *ipso facto,* become members by the payment of dues, as provided in Article III of the Constitution, but who have been proposed and seconded by active members of the Club who are not members of the Executive Committee. The

* Altered by amendment.

Committee shall, further, have power to decide all doubtful cases of eligibility.

II. A quorum of the Executive Committee shall consist of a majority of its members.

AMENDMENTS

I. Dues raised to $1.50 (Aug. 13, 1923).

II. Dues of Associate Members $3.00 (Sept. 1, 1924).

III. (Insert in Article IV) and an Assistant Treasurer. This officer shall be a resident of Woods Hole. His function shall be to collect dues from members and to perform such other duties as may be delegated by the Treasurer. (June 30, 1930).

IV. Employees of the M. B. L. shall be eligible to Associate membership in the M. B. L. Club, provided that their names are proposed and vouched for by two active members of the Club. Furthermore, such employees are allowed membership at the reduced fee of $1.50, and such membe.s are allowed all the privileges of the Club, though they may not vote at its meetings. (By the Executive Committee, 1933).

V. The Executive Committee shall consist of nine members and shall include the officers of the Club and the Chairmen of the House and Social Committees.

The duties of this Committee shall be to attend to such general affairs of the Club as the running of the Club-House, and the appointment and discharge of a House Committee, a Social Committee, and such special Committees as it may deem necessary. (Aug. 4, 1916).

IMPETIGO

Dr. David Cheever
Associate Professor of Surgery, Harvard Medical School

Uncleanliness is a term distinctly objectionable to people of refinement, and yet uncleanliness in the summertime is frequent among just this group —unintentionally so, of course.

Summer weather demands a different type of hygiene from that required by winter conditions. All biologically inclined people know that there is a difference in the types and luxuriance of flora in culture test tubes depending on whether they are kept cooled or incubated; and all housekeepers know how much more readily the contents of the bread box will mold in the summer than in the cooler months. These same principles apply to the care of the human skin, and yet mothers who will see that their children are bathed carefully at home in the winter will allow them to go for days in the summer, relying solely on the very doubtful cleansing effects of cold salt water without soap. These same children who have fresh clothing every day in the city, wear playsuits at the beach for days without change, and bathing suits which are rarely thoroughly washed and are frequently borrowed more or less indiscriminately.

In most cases these practices are not harmful, but now and then a wound in the skin will occur and, in an environment of perspiration and soiled clothing, a slight infection may start from organisms which ordinarily live harmlessly in and on our skins in small numbers, but under conditions favorable for rapid multiplication, they may cause a condition known as impetigo. Once this skin trouble has appeared it is a genuine problem, though rarely, if ever, a serious one, but very persistent unless correctly treated. It can spread rapidly to other parts of the same person and almost as readily to other persons. It occurs most commonly on the exposed parts of the body but may be transferred, especially by the fingers, to the covered parts.

The first stage of impetigo is marked by tiny blisters, extremely superficial, which break almost immediately and form golden or brownish crusts under which serum is constantly poured out, spreading to form larger and thicker crusts. The conditions under these crusts are ideal for rapid growth of the organisms, and the application of ointments outside the crusts makes these conditions still more favorable and increases the severity of the impetigo.

The proper treatment consists of the careful removal of the crusts with soap and water on a bit of gauze or paper tissue which must be burned; then the careful drying of the spots with fresh tissue to be immediately destroyed; finally, the application of very mild antiseptics. As a rule, ammoniated mercury ointment in the strength of not over five per cent. will produce best results; strong ointments, strong soaps, and the use of alcohol will usually so stimulate oozing that further spreading will occur and delay healing. The fingers must be kept absolutely away from the spots and fresh pillow-slips used every night, and the use of all towels and face cloths forbidden because of the easy spread to other surfaces. On a man's bearded face it is generally necessary to omit shaving for three or four days since the razor spreads the germs in spite of the greatest care. As is obvious, every person with impetigo should practice careful individual personal hygiene. Isolation such as is required by the "children's diseases" is not necessary but is helpful and perhaps not unreasonable, inasmuch as proper treatment applied for three days should completely cure impetigo.

To Present "You Never Can Tell," Shaw Drama, at M. B. L.

"You Never Can Tell", an early four-act drama by Bernard Shaw dealing with that perennial issue of Shaw's, the rights of women, will be presented in the Marine Biological Laboratory auditorium Monday evening by the Penzance Players, for the benefit of the **Collecting Net** scholarship fund.

The Players are to be remembered, especially for their production of "The Queen's Husband" by Robert E. Sherwood in the summer of 1931, and "Meet the Prince" by A. A. Milne last summer. These were given on Penzance Point at the residence of Mr. and Mrs. J. P. Warbasse, so this year's Auditorium performance is a unique venture. The present production is under the direction of Mrs. Dorothy Baitsell who has had many years experience in Little Theatre work in New Haven, Connecticut.

In the cast are several old favorites, besides some newcomers to Woods Hole drama. **Alfred Compton**, of Princeton University Theatre Group, who takes the Shavian role of the waiter, will be remembered for his excellent performance of the leading part in the "Queen's Husband", and his Mr. Battersby in "Meet The Prince". **Tommy Ratcliffe,** of the Harvard Dramatic Association, who played the bombastic general in 1931, and the leading role of the imitation prince in last season's production, will play Valentine, the Dentist "Duellist of Sex" in "You Never Can Tell". **Vera Warbasse** of the Connecticut College for Women, who has taken important roles in the preceding performances plays the part of Gloria—the "Woman of the Twentieth Century," Mrs. Clandon, her mother, old fashioned champion of Women's Rights movements, is to be played by **Peggy Clark** of Smith College, who was seen last season in the character part of the overbearing Mrs. Faithful.

Fred Copeland, of Williams, who has taken part in the other plays is the eminent Queen's Councillor. Mr. Bohun who undertakes to straighten out all difficulties of plot.

In addition to these old players, four new ones have appeared. **Eric Warbasse** will play Mr. Crampton, husband of Mrs. Clandon. **Bol Giddings** takes the part of McComas the solicitor, and **Faith Adams** of Vassar and **Manton Copeland** of Williams are the talkative twins—Dolly and Phil.

Mrs. George A. Baitsell is the director, and Robert Chambers, the stage manager. Wister Meigs is in charge of the settings, of which there will be three in the course of the four acts. Maynard Riggs is property man, and James Sever electrician.

Reserved seats are on sale at one dollar at the Collecting Net office, the M. B. L. office and Robinson's Pharmacy in Falmouth, and other tickets, at fifty cents, will be purchaseable at the gate.

St. Joseph's Church Benefits From Musicale Last Monday

The visiting Knights of Columbus choir from New Bedford, supplemented by local and visiting soloists, presented an evening of entertainment, mostly musical, for the benefit of St. Joseph's church Monday evening at the Marine Biological Laboratory auditorium.

The choir, the entire glee club of the McMahon Council of the Knights of Columbus, opened the program with Houssian's Messe en l'honneur de St. Paul, in the conventional four parts, Kyrie, Sanctus, Benedictus, and Angus Dei, and contributed groups of English selections, with the director, John T. Curry, Jr., leading. Miss Carolina Finni, lyric soprano sang a group of Italian and German selections, including an aria, 'Donne Vaghe' from Paisiello's opera, "La Serva Padronna," and Miss Gertrude Tripp, lyric soprano, several shorter English numbers. Male vocalists were Mr. James Evans and Mr. Leonard McDonnell. The instrumental portion of the program featured violin solos by Mr. Robert SanSouci, former Lawrence High School concertmaster, a member of the Boston College quartet, and Miss Helen McKenzie, a flute solo by Mr. Robert L. McKenzie, and a group of piano numbers including Rubenstein's famous "Kammenoi Ostrow," by Mr. Harry Bowker. With such a varied program, numerous accompanists were used, including Miss Mary Louise Stockard, Mrs. Maud Marceau Powers, Miss Joan Pecheux, and Mrs. Gladys Howard.

The succession of musical selections was interrupted about the middle by a farcical playlet entitled "Just a Few Laughs," with the following cast of characters:

His Honor, the Judge..........James Evans
Patrolman ....Edward Doyle
Mr. Casey ....Walter Considine
Mrs. Casey ....Mrs. James Evans
Mr. Margolis ....Tom McConnell

THE COLLECTING NET

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THE BERMUDA BIOLOGICAL STATION FOR RESEARCH (Part II)

J. F. G. WHEELER
Director of the Station

During 1932 there were forty-one visitors staying at the Station for periods varying from a few days to three and four months.

The following scientists carried out work there:

Prof. Karl Sax, Bussey Inst. Harvard (Plant cytology).

Prof. E. M. East, Bussey Inst. Harvard (Physiological work on Valonia & Halicystis).

Prof. B. White, State Antitoxin Lab. Mass. (Physiological work on Valonia & Halicystis).

Dr. H. R. Seiwell, Woods Hole Oceanographic Research ship "Atlantis" (Phosphate content of sea water).

Pierre Comte, Princeton (Geology).

Prof. J. P. McMurrich, Biol. Board of Canada, Toronto (Actinia).

Prof. B. R. Lutz, Boston Univ. (Physiological work on Ascidians).

Mrs. B. R. Lutz, Boston (Physiological work on Bufo).

M. B. L. Calendar

TUESDAY, AUGUST 1, 8:00 P. M.

Seminar: Dr. Conway Zirkle: "The effects of fat solvents upon the fixation of mitochondria."

Dr. A. W. Pollister: "The cytology of amphibian tissues."

Dr. B. M. Duggar and Dr. A. Hollaender: "The irradiation of biological suspensions by monochromatic light."

Dr. C. C. Speidel: Motion pictures showing some varieties of nerve irritation, as seen in living frog tadpoles.

WEDNESDAY, AUG. 2, 8:00 P. M.

Lecture: Dr. O. E. Schotte: "Organizers and inherent potencies in the embryonic development of Amphibians."

FRIDAY, AUGUST 4, 8:00 P. M.

Lecture: Dr. August Krogh: "Conditions of Life in the Depths of the Ocean."

SEX DETERMINATION IN HYMENOPTERA

DR. P. W. WHITING
Professor of Zoology, University of Pittsburgh

The problem of sex determination in the bee has excited interest from early times. Eventually the question seemed to be solved by the knowledge that a haploid set of chromosomes resulted in a male, a diploid set in a female. With the development of the idea of genic balance, however, it became clear that this explanation was inadequate and in 1925 Bridges wrote, "sex determination in the bee is the outstanding unsolved puzzle, although before the development of the idea of genic balance it seemed one of the clearest and simplest of cases." The inadequacy of the old explanation became very obvious when Bridges (1925b) reported that haploid tissue in Drosophlia appeared to be female and when the author discovered that diploid males in the parasitic wasp, Habrobracon, showing no traces of intersexuality, occasionally appear among the progeny of mated females.

Goldschmidt (1920) (*Continued on Page* 121)

TABLE OF CONTENTS

Photographs by Miss M. L. Russell

HARBOR and TOWN OF ST. GEORGE, BERMUDA

The Biological Station is on the horizon in the middle distance.

Dr. M. Reid, Boston Univ. (Physiological work on Bufo).

E. B. Benson, Boston Univ. (Physiological work on Holothuria).

Prof. L. V. Heilbrunn, Pennsylvania Univ. (Toxopneustes).

Dr. R. L. Biddle, Coll. New York (Tunicates).

Dr. T. C. Barnes, Yale Univ. (Physiological work on Crustacea & Algae).

Prof. E. G. Conklin, Princeton (Asymmetron).

Dr. R. Meader, Yale (Nerve tract degeneration studies in Fishes).

Prof. E. S. Goodrich, Oxford (General zoology, Asymmetron and Polychaet worms).

Mrs. Goodrich, Oxford (Parasitic protozoa).

F. Gilchrist, Harvard (General zoology and physiological work on Ligia).

F. Torrey, Harvard (Ecology of land Mollusca).

Prof. E. L. Mark, Harvard (General zoology, Odontosyllis).

Miss F. Felin, Scripps Coll. Calif. (General zoology, Fish eggs & larvae).

Dr. H. Richards, U. S. Nat. Mus. (Coll. Marine Mollusca).

Prof. R. B. Goldschmidt, Kaiser Wilhelm Inst. Berlin (General Zoology).

Dr. W. Beebe, N. Y. Zool. Soc. (Ecology & life history of shore and deep sea fishes).

J. Tee Van, N. Y. Zool. Soc. (Ecology of fishes esp. shore fishes).

Mrs. Tee Van, (Artist).

Miss G. Hollister, N. Y. Zool. Soc. (Shore and deep sea fishes esp. osteology of tails).

Miss J. Crane, N. Y. Zool. Soc. (Shore and deep sea fishes esp. scales).

Miss E. Van der Paas, (Artist).

V. Palmer, N. Y. Zool. Soc. (Shore fishes).

Dr. G. M. Smith, Sch. Med. Yale (Lateral lines and regeneration of melanophores in fishes).

H. Antz, Sch. Med. Yale (Lateral lines and regeneration of melanophores in fishes).

Prof. E. Newton Harvey, Princeton (Physiological work on Echinoderm eggs with centrifuge microscope).

Mrs. E. B. Harvey, Princeton (Physiological work on marine eggs with centrifuge microscope).

GENERAL COLLECTING

Many localities have been examined in the Reach, Castle Harbour, St. George's Harbour and round the shores of the islands and islets in the vicinity to ascertain the nature of the fauna and flora and to gain knowledge of the best collecting grounds for the more abundant forms at different times of the year. A record has been kept of the trips of the collecting launch, with the forms obtained, notes on spawning animals and any peculiarities in distribution that have been noticed. This survey has, of course, been done mainly in connection with the work of the visiting scientists

who have in many cases identified specimens of the groups in which they were interested.

Features of special interest were the wide distribution of the alga *Valonia macrophysa* and the discovery of *Halicystis* growing in Castle Harbour; a good collecting ground for the white sea-urchin *Hipponoe esculenta* and the discovery (by Prof. E. N. Harvey) that eggs and sperm of this form are mature in December while those of *Toxopneustes variegatus* are 80 per cent. ripe in June (Prof. Heilbrunn); the recurrence of ripe specimens of *Asymmetron* during the first week in August (Prof. Goodrich) and the finding of the land Nemertine *Geonemertes agricola* under stones on the margin of the Parish Dump, St. George's.

For Dr. Lutz's investigations the giant toad, which does not occur on St. George's Island, was collected from the ponds of the Aquarium at Flatts by kind permission of Mr. Mowbray and in the marshes near Hamilton.

SCIENTIFIC CONSTRUCTION AND ADDITIONS TO EQUIPMENT

A tide pool has been constructed on the point between Ferry Reach and Richardson's Cove, a natural hollow being supplemented by a wall of stone and cement. This was originally intended as a fish pond, but mechanical difficulties in building the wall high enough to exclude the sea at all tides, together with the possibilities of the old fish pond, led to a change of plan. The pool forms a useful stock aquarium for invertebrates.

The old fish pond, which was used for many years as a convenient dump, has been cleaned and repaired, and is entirely successful.

In September, as the result of a recommendation of the International Hydrographic Conference at Monaco a standard automatic tide recording machine was delivered to the Station from the U. S. Coast and Geodetic Survey, Washington. A stone house was built to accommodate the machine on part of the sea-water supply pier. The tide pole was fastened near the house and three bench marks, set in cement beds for levelling purposes, were established. These were levelled to the Ordnance Survey mark on the far side of the Swing Bridge road by Mr. Cyril Smith of Bermuda. The machine has been in continuous operation from October 1st.

In the chemical laboratory a small fume chamber has been built into one of the corners and a solid stone pier has been set up to carry the fine balance.

Gas (Philgas) has been laid on in this laboratory from a double cylinder installation placed on a cement bed outside the building near the eastern steps.

Additions of apparatus include a Leitz binocular microscope and a microtome from Prof. Conklin and a monocular microscope from Dr. G. G. Scott which fill a very great need.

A series of plunger jars has been set up in the general laboratory for investigations upon small active organisms that would escape from the aquaria. These were used with success for post-

SHELVING, ROCKY SHORE OF LONG-BIRD ISLAND
One of the best collecting grounds near the Station.

BERMUDA BIOLOGICAL STATION FOR RESEARCH

Main Building, two cottages and part of grounds. There are seven additional buildings on the property. Long-Bird Island is beyond the Station and Castle Harbor is in the distance.

larval fishes by Miss Felin. Another set in the cool room under the South Verandah was used by Prof. Goodrich in his work on the larvae of Asymmetron.

A new pattern diving helmet made to the order of Prof. Conklin has proved very successful.

LIBRARY

More than two-thirds of the reprints and bound volumes in the library have been card-indexed under the author's name. This work was done by Dr. and Mrs. Wheeler, Prof. McMurrich and later Miss Gallaudet. A sufficient number of the 1600 reprint cases devised and ordered by Dr. Mark have been made up to carry the reprints, and new shelves have been erected in the two library rooms to carry the present books and papers. The completion of the card index is at present in the hands of Mr. Cutter, temporary librarian.

The library has grown rapidly thanks to Prof. Conklin, who has sent many papers, duplicate reprints and bound works and to Dr. Mark who has made presentation of series from his library including the Proceedings of the American Academy of Arts and Sciences, the Bulletin of the Museum of Comparative Zoology, Harvard, and the Publications of the International Council for Exploration of the Sea. Lately Dr. Mark has sent a great many reprints and works dealing with plankton for which I personally am very grateful since plankton literature was practically non-existent previously. The Station is indebted to the Smithsonian Institution for the Proceedings of the U. S. National Museum and other publications, to Prof. E. Newton Harvey for arranging with the Wistar Institute of Anatomy and Biology for a year's subscription to the Journal of Cellular and Comparative Physiology, and to Dr. W. T. Porter who has presented back numbers to September, 1928 and the current issues as they appear of the American Journal of Physiology. Mr. Mowbray, Director of the Bermuda Aquarium, has turned over to the Station a

"BOILERS" OR DIMINUTIVE ATOLS OFF THE SOUTH SHORE, FRINGING REEF IN THE DISTANCE

complete set of the scientific results of the "Challenger" Expedition which is a valuable acquisition. The Trustees of the British Museum (Natural History) are presenting the "Great Barrier Reef" Reports as they are published. The National Academy of Sciences of the U. S. has presented a set of all of its publications that are now available.

Several workers have presented copies of their works and other gifts of this description have come from persons interested in the welfare of the Station.

Purchases for the library include five general works on life in the sea, a complete set of the "Nordisches Plankton" and the "Treatise on Zoology" edited by Sir. E. Ray Lankester in eight volumes.

SALE AND SUPPLY OF SPECIMENS

There has, of course, been no attempt at a supply department, but specimens have been requested by scientific workers in other countries which I have been glad to collect seeing in this matter a means of widening the interest in the Station. A request for Halicystis was dealt with by Dr. Blinks of the Rockefeller Institute Staff in Hamilton who sent the material to me for packing and shipment.

T. CUNLIFFE BARNES, *Yale University*

SUMMARIES OF THE WORK OF SOME OF THE VISITING SCIENTISTS DURING 1932

Series 1. *The All-or-None-Law.* Single fibre nerve-muscle preparations were made of a dozen species of Crabs to test the validity of the all-or-none law by electrical and natural stimulation of motor nerve fibres. It was found that this law does not hold for Crustacea. Paper to be published soon.

Series 2. *Origin of Kinesthetic Impulses in Crustacean Limbs.* By means of an amplifier these impulses were located arising from the muscles themselves. Other sources of stimulation such as the bristles and integument were eliminated. Paper to be published soon.

Series 3. *Space Orientation and Salt Requirements of Ligia.* Approximately 1000 isopods were studied in salt solutions and in respect to their ability to orient towards the sea or survive in air. Paper to be published soon.

Series 4. *Influence of Ice-Water on Marine Algae. Valonia, Halicystis* and *Acetabulum* were grown in sea water containing trihydrol. No definite results were secured with the first two forms. Further work is necessary to compensate for the dilution of the salt concentration.

E. G. CONKLIN, *Princeton University*

BREEDING PERIOD AND BEHAVIOR OF ASYMMETRON LUCAYANUM

Specimens of this interesting species occur in small numbers in the clean, bottom sand under water 10-20 ft. deep in a small area near Nonesuch Island. In 1931, between June 1st and August 1st, specimens were collected by dragging a bucket, weighted on one side, over this area and sifting the water and sand so taken through a fine wire screen. Animals were collected at various times during the day and evening and were kept for several days in dishes of clean sea water in the laboratory. They are hardy and will live for a week or more under such conditions. During the period mentioned no eggs were laid by the animals brought into the laboratory although the gonads were full and the spermatozoa active. Attempts to artificially fertilize the eggs failed, probably because the eggs were not in the precise stage necessary. After my departure from the Station on August 5th, 1931, my assistant, Mr. J. K. Donahue, found on August 14th a lot of early gastrulae in a dish containing animals which were collected on August 12th and which must have laid during the evening of August 13th. Some of these gastrulae were reared to the early larval stage.

In 1932 I collected specimens of Asymmetron between June 20th and July 11th but again was unable to obtain any embryological material during that period. However, Professor E. S. Goodrich found that eggs were laid and fertilized during the evening of August 1st, 1932, and many of these were reared to the larval stage with small, round mouth and first gill slit. Therefore, Asymmetron in Bermuda breeds early in August, although it is possible that it may breed also at other periods.

The marked asymmetry of the adult suggested that the animals might occupy an asymmetrical position on or in the sand, and in order to test this those captured were placed in small aquaria on a deep layer of the sand in which they were taken, but owing to their small size and transparency they could not be observed satisfactorily in such aquaria. Consequently a very narrow aquarium with a space of only about ¼ inch between the two glass sides was made and filled with sand to a depth of 4-6 inches and with an equal depth of running sea water above the sand. Even in so narrow an aquarium it was difficult to see them when they were buried in the sand and accordingly a still narrower aquarium was made with the glass sides only about ⅛ inch apart, and in this

the animals could be seen and photographed when buried.

Asymmetron, like Branchiostoma, remain inactive for long periods unless actively stimulated, when they race around violently for a short time only to relapse into a passive and apparently exhausted condition. Such passive individuals lie indifferently on their right or left sides, or when in narrow places on their dorsal or ventral sides, and when they were introduced into the narrow aquarium they would generally lie on the surface of the sand, unless they chanced to touch the sand head first or until the water was agitated, when they would burrow into the sand by an undulatory and more or less spiral movement. Once buried in the sand they would often lie passive for hours or even for days, but usually they would squirm to the surface of the sand and protrude the anterior or posterior end or come out of the sand altogether; sometimes both anterior and posterior ends would protrude and in few instances did they stand vertically in the sand with the anterior end and mouth exposed, as in Willey's ('94) figure of the Amphioxus at Messina. On the contrary they took and held almost any possible position, some with head up, others with head down, some parallel with the surface and others at an angle with it. Usually the body was curved towards the ventral side and sometimes it was thrown into sinuous folds. A few photographs of animals in these various positions were taken. The asymmetrical organization of the adult Asymmetron is not associated with any peculiar position which they assume when they are at rest either in the water or in sand.

F. Gilchrist, *Harvard University*

SALT REQUIREMENTS OF LIGIA

Lover's Lake, one mile west of the Station, was explored, and found to have a considerable tide and to be nearly, though not quite, as saline as sea water. The lake is an excellent collecting spot for a small viviparous *Synaptula*.

In collaboration with Dr. T. C. Barnes experiments were made on tidal-zone isopods, *Ligia*. These were found to be positively geotropic and phototropic and rheotropic. They will live in the laboratory on moist sand for periods up to twenty days, and will survive complete submergence in sea water for six days, but die quickly in a dry environment. They are remarkably tolerant of changes in the osmotic pressure of the fluids they are submerged in, surviving appreciable lengths of time in distilled water and sea water concentrated by the addition of dry salts. In working with solutions containing single ions, it was found that potassium was the most toxic of the metallic ions commonly found in sea water. Sodium, calcium and magnesium were less toxic. Some measure of the viability of the animals in the various solutions could be obtained before actual death by observing the rate of gill movements, which slowed down some time before actual death. In solutions containing only potassium ions there were no gill movements at all.

B. R. Lutz,
Boston University School of Medicine

THE EFFECT OF ADRENALIN CHLORIDE AND TOAD VENOM ON THE BLOOD PRESSURE OF THE TROPICAL TOAD, BUFO MARINUS

How *Bufo marinus* secretes and stores in its skin glands an enormous amount of venom containing powerful adrenalin and digitalis-like substances without harm to itself is not known. Various workers have determined the pharmacological action and the minimum lethal dose of toad toxin for animals other than the toad. They generally agree that the toad is relatively immune to its own venom. Abel and Macht (1912) found that both bufo-epinephrin and bufagin, isolated from the venom, when added to a Locke solution used to perfuse the blood vessels of *Bufo marinus*, caused vasoconstriction. Gunn (1930) found an adrenalin-like substance in the skin secretion of the South African clawed toad, *Xenopus laevus*, which produced striking circulatory effects in the cat, rabbit and guinea-pig. Neither adrenalin nor the skin secretion had an effect on the circulatory system of *X. laevus*.

No reference in the literature could be found concerning the effect of toad venom on the blood pressure of the toad, nor even concerning the measurement of the blood pressure in this amphibian. Beiter and Scott (1929) found that an intravenous injection of 0.2 cc. of adrenalin chloride, 1 in 10,000, gave a rise of blood pressure in the frog lasting one hour and a quarter. They found the systolic blood pressure in *Rana catesbiana* to be 32 mm. Hg. The present report concerns the effect of adrenalin chloride and the crude venom of *Bufo marinus* on the blood pressure in the same animal. A determination of the minimum amounts of these substances necessary to produce a rise in blood pressure on intravenous injection is also reported.

The fore-brain of *Bufo marinus* was destroyed and the spinal cord posterior to the second vertebra was pithed. The blood pressure was recorded by a mercury manometer from a cannula in the femoral artery. Injections were made through a cannula in the femoral vein.

The average systolic blood pressure in twenty-five animals was 30 mm. Hg., and the average

heart rate was 64 per minute. Adrenalin chloride, 0.2 cc. of 1 in 50,000 (0.004 mg.), caused a rise in systolic pressure from 60% to 100% of the original pressure and lasting from three to six minutes. Weaker doses, such as 0.2 cc. of 1 in 200,000 (0.001 mg.), gave up to 56% rise accompanied by a 20% fall in heart rate. The threshold dose of adrenalin chloride for blood pressure was 0.1 cc. of 1 in 1 million (0.0001 mg.).

The crude venom was expressed from the large gland behind the ear, weighed, dissolved in Ringer's solution and injected immediately. This gave invariably a rise in systolic blood pressure, the extent varying with the dose. For example, in one case 0.05 mg. of crude venom produced a 120% rise, and 0.13 mg. a 292% rise. The heart rate was usually decreased. The threshold dose of crude venom for blood pressure was 0.0006 mg. per 100 gms. of body weight.

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E. B. Benson, B. R. Lutz
Boston University

THE ACTION OF ADRENALIN AND CERTAIN DRUGS ON THE ISOLATED HOLOTHURIAN INTESTINE

In vertebrates the action of adrenalin on smooth muscle is generally the same as the effect of stimulation of the sympathetic supply to the same muscle; consequently both excitatory and inhibitory effects are observed. In invertebrates only augmentor actions of adrenalin have been described, with one exception. Wyman and Lutz (1930) found that adrenalin caused inhibition of tone, amplitude and rate of beat of the cloacal muscle of the holothurians, *Cucumaria frondosa* and *Stichopus badionotus,* Selenka (*S. moebii,* Semper). The intestinal blood vessel of *S. badionotus* however showed a marked acceleration of beat in adrenalin 1 in 100,000. By using other autonomic drugs Wyman and Lutz (1930) found that in the holothurian a fairly complicated schema of drug action exists similar to that which forms the basis of much of our reasoning concerning the nature of autonomic innervation in higher and presumably more complex animals. The present report deals with the action of adrenalin and other drugs on the intestinal muscle of *S. badionotus.*

Adrenalin chloride solution (Parke, Davis & Co.) added to the sea water bath (to make 1 in 50,000) in which a ring of the holothurian intestinal muscle was suspended caused a rise in tonus and sometimes in amplitude. The threshold dose was 1 in 125,000. After atropine (1 in 8,000) the usual adrenalin response was reversed or prevented.

Pilocarpine nitrate (Merck) 1 in 500 to 1 in 1000 gave a rise in tonus and an increase in amplitude.

Physostigmine salicylate (Merck) 1 in 10,000 to 1 in 1 million gave a marked rise in tone. The amplitude was generally increased or unaltered, but when the tonus effect was extreme, a decrease in amplitude occurred.

Atropine sulphate (Merck) 1 in 8,000 or stronger caused inhibition of tone and amplitude and sometimes cessation of beat.

Mechanical stimulation of the ring of intestinal muscle either by touching, or by squirting the fluid against it in the bath produced an immediate temporary contraction easily distinguished from the drug effect.

Chloretone in a concentration present in adrenalin chloride 1 in 50,000 was without effect.

It appears, therefore, that the usual antagonism found in the vertebrate between atropine and the parasympathetic stimulants, physostigmine and pilocarpine, exists in the holothurian intestine. While the motor effect of adrenalin on the holothurian intestinal muscle is the reverse of its effect on the cloaca in the same animal, this is not without counterpart in the vertebrates, since Lutz (1931) found adrenalin to be motor to the stomach and inhibitory to the posterior end of the spiral valve and rectum in elasmobranchs.

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Marion A. Reid
Boston University School of Medicine

PHYSIOLOGICAL PROPERTIES OF THE LYMPHATIC HEARTS OF BUFO MARINUS

The lymph hearts are normally under the control of spinal cord centers, but have been reported capable of beating after denervation since the time of this discovery (1796 or 1832). Many investigators believed that this extra-spinal beating was due to accidental causes, drying or injury currents. Of course there was always the possibility that the nerve had regenerated. Last spring Dr. Pratt discovered that the lymph hearts, anterior and posterior, of the same side were always synchronous. The same synchronism was found in the toad. In *Bufo marinus* I denervated an anterior lymph heart and then after a period of

ten days took a record of its activity simultaneously with that of the homolateral posterior organ. In the group of toads studied in Bermuda I always obtained synchronous and therefore neurogenic beating.

Because of the previous doubtful myogenic beating found in the frogs studied by the denervated-*in-situ* method or by the use of isotonic salt solution baths, I tried transplantation. The retrolingual lymph sac was chosen because of its transparent membrane and its easy accessibility. Within ten days most of these transplanted lymph hearts were beating. Furthermore, the organs were large enough to permit the taking of kymograph records. Such records supported previous observations to the effect that electrical stimuli, single or faradic, may increase the rate of the myogenic lymph heart contractions but cannot tetanize it. There had been a change from a skeletal muscle type of response to a cardiac type. Two experiments showed that curare did not cause abrupt cessation of beat as in the normal, but rather an initial stimulation which was followed by continued activity when there was ample blood supply.

To make this myogenicity unquestionable it was necessary to show that there were no nervous elements present. I tried a few vital methylene blue preparations at the Station, but brought most of my transplants back here where I have used Rogers' silver impregnation method (suggested by Dr. Meader). Apparently there are no ganglia in either the normal or transplanted organs. The influence of possible vaso-motor nerves was obviated by the fact that one very *active* transplant floated absolutely free in the lymph sac.

The manuscript of the paper that I have outlined above will be finished soon. Other observations which I have made on the normal neurogenic beating of the lymph hearts, especially those on the location of the spinal centers, will probably be incorporated into another paper on synchronism.

T. W. Torrey, *Harvard University*

ECOLOGY OF LAND MOLLUSCA

During a five week period, from late July through August, an ecological study of land molluscs was successfully carried on. This entailed extensive collections and detailed notes on all those factors believed to bear on the problem. The greater part of the work was intentionally limited to St. George's Island. It was believed that a tremendous advantage could be derived from a detailed introductory study of a relatively small area. One thus obtains a real basis for comparison with other more distant regions, but at the same time profits by shorter visits to them, the previous discipline furthering a rapid appreciation of the essential points involved. With this in mind, St. George's Island was carefully examined, and then, as far as time permitted, the observations were extended to smaller and more distant islands.

The collection of forms, both snails and slugs, runs into the hundreds and includes probably all the living species. Of the ecological factors, vegetation, soil, other organisms, climate, topography, etc., special attention was centered on the first three.

A report of the findings and conclusions derived therefrom will appear at a later date. Following a careful check on the identifications, the shells will be turned over to the laboratory for its permanent collection.

Karl Sax, *Harvard University*

CYTOLOGICAL INVESTIGATIONS OF CERTAIN SEMI-TROPICAL PLANTS

The work at the Bermuda Biological Laboratory was confined largely to a study of chromosome number and morphology in the plants available on the island of Bermuda. The native species are not of much interest for such studies, but a number of introduced species were studied and provided some interesting material.

The common Aloe was found to have four large pairs of chromosomes and three small pairs. This chromosome complex is exactly the same as found in the related genera Gasteria and Haworthia. In this case the cytological analysis is in harmony with the taxonomic classification. The work on Aloe will be included in Mr. Marshak's study of this family.

Last summer one of my students made a study of several species of Yucca and found that this genus has 5 large pairs of chromosomes and 25 very small pairs. The general appearance of Agave would indicate that it might be related to Yucca, but the taxonomic classification places Yucca in the family Liliaceae, while Agave is in the family Amaryllidaceae. A cytological study of *Agave americana*, which is abundant in Bermuda, shows that the chromosome size and number is exactly the same as in the genus Yucca. The cytological situation is so unusual in these plants that the same numbers and sizes in the two genera must mean that they have had a common origin and are rather closely related, even though the taxonomic characters have caused them to be placed in different families. It seems clear that the taxonomic grouping in this case is artificial and does not represent the phylogenetic relationships. The two genera are found only in the

southern part of North America. A report of this work will be published in the Journal of the Arnold Arboretum.

The chromosome number in *Carica papaya* was found to be 9. Although the sexes are separated in this genus, there was no evidence of heteromorphic pairs or sex chromosomes.

G. M. Smith

Yale University School of Medicine

1. Inflammatory reactions associated with healing of wounds were studied at various stages in a number of Bermuda fishes. In these studies particular attention was given to the rôle of the pigmented cells, their development, function, fate and relation to other cells found in the inflammatory processes.

2. Along somewhat similar lines a study was made of the repair of wounds in *Holothuria rathbuni*.

3. Certain physiological experiments on the lateral line of fishes, already begun at Yale University and the New York Aquarium, were extended by observations on a considerable number of Bermuda fishes. These experiments consisted in testing the intake into the canals and the subsequent outflow from canals of artificially colored fluids. The experiments confirmed the impression that the lateral line canals of the head and body of fishes function in part at least as a testing mechanism for chemical or physical changes of surrounding water.

SEX DETERMINATION IN HYMENOPTERA

(*Continued from Page* 113)

postulated that the egg cytoplasm has a tendency toward maleness which dominates the female tendency of a haploid nucleus but which is dominated by the doubled female tendency of a diploid fertilized nucleus.

Schrader and Sturtevant (1923) suggested an algebraic sum hypothesis with female tendencies of the haploid set inadequate to swing the balance, while the genetically similar set, doubled by fertilization, results in a female.

It is generally agreed, however, that none of the hypotheses is adequate and we must seek something more than mere quantitative or numerical difference between chromosomes of males and females in Hymenoptera.

In Habrobracon, females heterozygous for one or more traits occasionally produce haploid mosaic sons which show certain characters in one part of the body and allelomorphic ones in the other. In studying these males it has been found that several traits, in structure as well as in color, are not autonomous. In other words the recessive part of the mosaic may be influenced by the dominant allelomorph in adjoining tissue, as discovered by Sturtevant for vermilion in Drosophila.

One interesting combination of colors occurring in mosaic eye of Habrobracon will illustrate this. White and ivory are non-allelomorphic recessives, each causing the eye to be white. From a female heterozygous for both (Whwh-Oo^{i}) one section of the compound eye of the mosaic son may be genetically white, the other genetically ivory. Were these both autonomous we would expect such an eye to be uniformly white. Such is not the case however. The white non-ivory region remains white and is sharply marked off from the ivory non-white region which is black at the border. The double dominant character is reconstituted in the region where the two recessives are in contact although there is no diploid tissue there. This appears to be accomplished by a diffusion of something from the white region into the ivory.

Such a reaction has been discussed in some detail since a similar type of behavior in mosaic males has led to the formulation of the theory to be presented.

Study of the external genitalia of mosaic males reveals that many show feminization close to the line where the genetically different tissues meet. A sensory appendage or even a sting may occur. These organs are characteristic of females only and are never found in non-mosaic males. The condition is particularly striking when the males are mosaic for honey body color. The fact that many of these males are from virgin mothers proves that the feminized region does not develop from a fertilized diploid nucleus. The female structures always occur on one side of the midline only, therein resembling the reconstituted dominant black eye color mentioned above, and suggest that some influence has been exerted by one type of haploid male tissue on another adjoining.

These feminized structures do not appear on all mosaics for in many the line of mosaicism does not pass through the genitalia. Neither do they occur in all mosaics where the line does pass through the genitalia. These facts led to the supposition that there are in Habrobracon two kinds of males, genetically distinct for sex-determining factors but phenotypically similar. When tissues differing in these sex-determining factors adjoin

in a haploid mosaic male, one influences the other and there result traits characteristic of diploid tissue, in other words, female.

Of the two types of males postulated, one contains 1 X chromosome + 1 set of autosomes and, in its X chromosome, the genes F and g; the other contains 1 Y chromosome, + 1 set of autosomes and, in its Y chromosome, the genes f and G.

Females contain 1 X + 1 Y + two sets of autosomes, being heterozygous for factors F and G (F.g/f.G). Segregating eggs would result in two kinds of males in equal numbers from a virgin mother.

In the reduced egg there are four oötid nuclei, two being 1 X + 1 set of autosomes and two 1 Y + 1 set of autosomes. Normally three of these degenerate as polar nuclei in the peripheral cytoplasm. It is supposed that if an X-bearing sperm enters the egg there is selective fertilization, so that it fuses with a Y-bearing egg nucleus. Similarly if the sperm which has entered be Y-bearing, it will fuse with an X-bearing egg nucleus.

Diploid males would result when an X sperm unites with an X egg or Y with Y. They would be 2 X + 2 sets of autosomes or 2 Y + 2 sets of autosomes and would have the same genic balance as normal haploid males. It has been shown by genetic tests that maleness in fertilized eggs is determined at or shortly after fertilization.

This hypothesis of sex-determination in Habrobracon is consistent with results of genetic and cytological studies. It is, moreover, highly suggestive for explaining the known facts of the life histories of other Hymenoptera, such as alternation of generations, polyembryony, and production of females parthenogenetically. Finally, it is consistent with the theory of genic balance on a ratio basis, and, whether it ultimately proves true or not, may serve as a working hypothesis stimulating new modes of attack.

The facts and principles upon which the theory of sex-determination here reported is based have been brought to light by investigations which have been generously supported in recent years by grants from the Committee on Effects of Radiation on Living Organisms (National Research Council).

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(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 25.)

THE BIOLOGICAL LABORATORY

COLD SPRING HARBOR

STREAMING POTENTIAL MEASUREMENTS

D. R. BRIGGS

When a liquid is caused to flow through a fixed diaphragm, membrane, or capillary tube there is set up an electrical potential difference in the system in the direction of the pressure gradient which is a function, among other factors, of the magnitude of that gradient and of the electrokinetic potential existing between the movable and immovable layers of ions tangential to the pore walls. To the current derived from such a potential, Beetz[1] gave the name "Strömungströme" from which the name "streaming potential" has been adopted to designate the potential thus formed. Early studies of this phenomenon were made by Quincke[2], Zollner[3], Edlund[4], Haga[5], Dorn[7], and Elster[8], the general observation being that the potential obtained was directly proportional to the pressure gradient applied. The phenomenon was ascribed as due to a kind of electrical convection, and to be the result of the electrified layer of the liquid carrying its charge along with it as it moves through the capillary. This results in a difference of potential becoming manifest at the two ends of the tube, which is compensated, if by no other means, by electrical conduction backwards through the column of liquid in the tube.

Helmholtz[9] in his well known paper on interface potentials, first treated the subject mathematically. The following derivation of the streaming potential equation is that given by him.

He considers the simple case of a single capillary filled with a liquid which is being forced through the tube under constant pressure and across the interface, solid-liquid, at which there exists a potential difference. Let (**e**) denote the electrical density of the movable layer at a distance (x) from the wall of the tube. The value of the velocity of motion (v) of the liquid at the wall of the tube being zero, then the value of (v) at the distance (x) will equal $v = \partial v \partial x \cdot x$. The amount of electricity which is carried along by the liquid in unit time in the surface element $ds \cdot dx$ then will be $I_s = e \partial v \partial x \cdot x : ds \cdot dx$. Integrating a part of the expression through x —

$$\int e \cdot x \cdot dx = -\tfrac{1}{4\pi} \cdot \int_0^\infty \partial^2 \phi / \partial_x \, z \cdot x \, dx$$

$$= \tfrac{1}{4}\pi \, (\phi_i - \phi_a) = \tfrac{1}{4}\pi \cdot \zeta$$

which, when the dielectric constant, D, is considered (following Perrin's[10] suggestion), becomes the same value as that obtained from consideration of the two oppositely charged layers as forming a condenser, i. e.,

$$e\,x = \frac{\zeta D}{4\pi}$$

where ζ is the P.D. across the interface. This is the "same value which the moment of the double layer would form if the opposing electricities of the system were all concentrated into the interface. Thus it results that the entire cross section of the tube of liquid carries along an amount of electricity in unit time"

$$I_s = \zeta D/4\pi \cdot \int \partial v/\partial x \cdot ds$$

From Green's Theorem

$$\int \partial v / \partial x \cdot ds = -\int\int \Delta v \cdot dy \cdot dz$$

$$= -\frac{P\,A}{\eta L}$$

where P is the pressure difference between the two ends of the tube; L is its length; A, its area of cross section; and η, the viscosity constant of the liquid. This relationship holds only in the case where the flow of liquid is not turbulent flow, i. e., when

$$\frac{\partial v}{\partial h} = 0$$

and v is a function only of y and z, where h is the dimension parallel to the axis of the tube and y and z are the dimensions at right angles to this axis. This is the type of flow for which Poiseuille's Law holds. In a diaphragm where Poiseuille's Law does not hold, therefore, the streaming potential equation fails, also.

Collecting the terms of I_s, then, —

$$I_s = -\frac{P A \zeta D}{4\pi \eta L}$$

An electromotive force E, is thus created between the ends of the tube and the amount of

electricity (I_A) which is conducted back through each area of cross section (A) in unit time will be

$$I_A = \frac{E A}{R L}$$

where (R) is the specific resistance of the liquid.

If there is no other means of conductance than that through the column of liquid, an equilibrium will be established when

$$I_S + I_A = O$$

then

$$\frac{E}{R} = \frac{\zeta P D}{4 \pi \eta} \text{ or } E = \frac{\zeta P D}{4 \pi \eta \lambda}$$

This equation shows that the E. M. F. (E) observed when a liquid is forced through a capillary across the ends of which there is a difference of hydrostatic potential (P), is directly proportional to (P), to the potential difference across the interface solid-liquid (ζ), and to the dielectric constant (D) of the liquid. (E) is inversely proportional to the viscosity (η) of the liquid and to its specific conductivity (λ), but is *independent* of the *dimensions of the tube or diaphragm,* so long as the diaphragm material has *no conductivity in itself.* This equation was modified by Lamb[11] to include a term defining the slippage of the liquid layer along the face of the solid. However, this slippage has been considered so small as compared with the relative motion of the liquid over the film of liquid which is in contact with the solid, that it is negligible. A recent treatment of the subject from the standpoint of the ionic theory (unknown in the time of Helmholtz) and the diffuse double layer theory of Guoy[12] has led Bikerman[13] to virtually the same equation for the streaming potential as that given above as a first approximation. One important modification is the substitution of the value ($\lambda + \lambda_2$) in place of simply λ. Of these two factors in the specific conductivity, λ signifies that of the liquid in bulk and λ_2 is the surface conductance. $\lambda + \lambda_2 = \lambda_S$ which is discussed below, λ_2 may be either positive or negative. Also, according to Bikerman's treatment of the subject, the above equation may not hold if the size of the pore is very small, or the electrolyte concentration is very low.

As a means for measuring, ζ, the electrokinetic or interfacial potential, the streaming potential method has some advantages over the other more commonly used methods, i. e., electrophoresis and electro-osmosis. It has serious disadvantages, also The method best suited to the particular material to be investigated will depend upon the nature of that material and upon the conditions under which the measurement of ζ is to be made. Only those materials which can be made into a capillary tube, or pressed into the form of a diaphragm, or which are sufficiently surface active to completely mask the surface of such a diaphragh, can be investigated by the streaming potential method. The solutions, or pure liquids, against which the ζ-potential is desired cannot have a specific conductivity greater than 1 x 10^{-3}mhos, corresponding to a HCl soln. of about .0025N. With conductivities greater than this figure, the observable streaming potential at pressures under one atmosphere become too small to be reliable. Higher pressures than one atmosphere should not be used in most cases because of changes they may cause in the dimensions of the diaphragm or because turbulent flow of the fluid thru the diaphragm may result, in which case the streaming potential equation no longer holds good.

Older experiments cited by G. de Villemontée[14] stated that with glass capillaries and solutions of copper, zinc and nickel sulfates, there was no streaming potential. The solutions used in these experiments contained 10 grams of salt per liter, however. Their conductivities were too high to allow any observable E. M. F. to be set up.

The principle advantage of the streaming potential method for determining ζ lies in the fact that no extrinsic current is passed thru the system, as in the case of cataphoresis and electro-osmosis, with the accompanying danger of electrolytic formation of ions other than (or in different concentrations to) those known to be in the system. The composition of the solution passing thru the diaphragm can always be known, therefore, more accurately. The fact that the dimensions of the pores of the diaphragm do not enter into the equation serves to simplify greatly the measurements required to define ζ. From the streaming potential equation for ζ, i. e.,

$$\zeta = \frac{E \lambda}{P} \cdot \frac{4 \pi \eta}{D}$$

it is seen that in dilute aqueous solutions where η and D may be considered constant, three factors have to be measured simultaneously, in order to define ζ. These are P, the pressure gradient across the diaphragm, E, the streaming potential set up across the two ends of the diaphragm, and λ, the specific conductivity of the liquid being forced thru the diaphragm.

The value, P, is easily obtained, E and λ, however, may offer some difficulty. E is best measured by using some capacity instrument such as a quadrant electrometer or ballistic galvanometer. With such instruments very small amounts of

current need pass thru the electrodes in order to charge the instrument. In this way polarization of the electrodes is practically eliminated. This chief source of trouble in the measurement of the streaming potential, E, can also be eliminated by the use of non-polarizing electrodes in some cases.

The value for λ, can be taken as that of the liquid in bulk, in cases where the streaming potential is being measured at interfaces where no appreciable surface conductance exists, but in most cases where a diaphragm of powdered material is being used, this value of λ cannot be used, i. e., the value for ζ so obtained would be incorrect, because the specific conductivity, λ_s, of the liquid within the pores of the diaphragm is greater, in such cases, than that of the liquid in bulk. A special procedure must be used in this case in order to estimate λ_s.

Figure 1 shows the streaming potential apparatus used by Briggs[15] in measuring the ζ-potential at the interface between various powders fibers, and aqueous solutions. The apparatus, with some modifications, has been used by Martin and Gortner[16] and Jensen and Gortner[17] to measure the value of ζ between dry cellulose or Al_2O_3 and various organic liquids, and by Bull and Gortner in numerous experiments which will be mentioned below.

As a source of pressure, a tank (T) of about 50 liters capacity was used. Air was pumped into this tank until the desired pressure was attained. This air was passed through washing bottle (Bo) and into the reservoir (R) which contained the liquid which was being forced through the diaphragm. Pressure on the surface of the liquid in (R) was measured with the mercury manometer (M). To obtain the value for the pressure (P) on the diaphragm, the pressure, due to the water column between the surface of the liquid in (R) and the point of emission of the liquid from the cell, had to be subtracted from the manometer reading. Stopcocks, S_1 and S_2, were used to release the pressure from the tank and the reservoir, respectively. Stopcock, S_3, could be used to stop the flow of the liquid through the cell when desired. S_4 was needed to allow air to escape from the first chamber of the cell when this was being filled with liquid.

The cell used was a combination conductivity cell and streaming potential cell. It consisted of

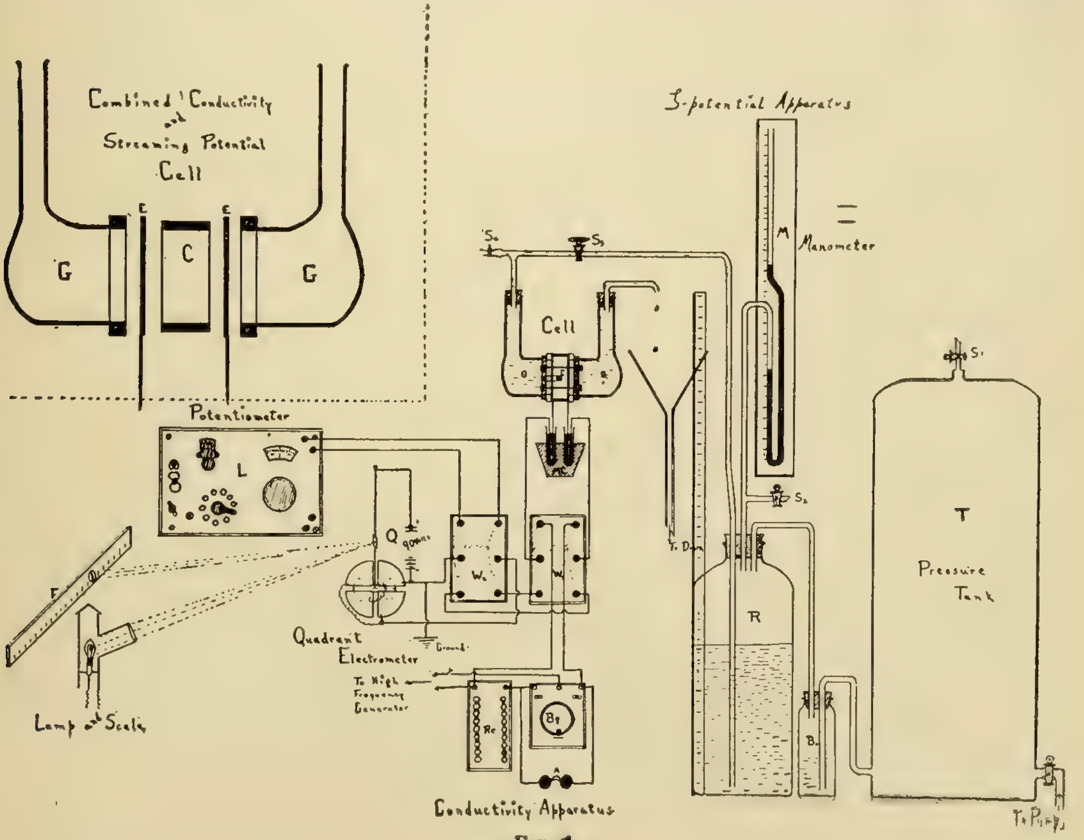


FIG 1

a glass center compartment (C) into which the diaphragm material was packed, two perforated gold electrodes (E), and two glass end compartments (G) with heavy glass flanges fitting up against the electrodes and which served as supports for a clamp which held the whole cell tightly together. In order to make the cell water tight, thin rubber washers cut from dental dam rubber were placed between the electrodes and the glass, care being taken that these did not cover any of the surface of the electrode which should be exposed to the contents of the chamber (C). The electrodes were disks made of 14K gold, one millimeter thick and 45 millimeters in diameter. The portion of the electrode which was exposed to the center compartment (C) was perforated with one millimeter holes as thickly as possible without appreciably lowering its strength.

At a point on the circumference of each gold disk was soldered a platinum wire which would dip into mercury cups (MC) and from which contact could be made with the electrical measuring equipment. Facility with which measurements could be made was increased by the use of two six-point double-throw switches (W_1 and W_2). To insure perfect contact in the switches, mercury cups and copper rockers were used. With switch (W_1) the cell could be connected either with the conductivity apparatus or the potentiometric apparatus. It is necessary to place a grounded copper shield around all the electrical parts of the apparatus to prevent stray body capacities from influencing the quadrant electrometer, Q, which was used to measure H. This instrument is a capacity potentiometer and requires an almost infinitesimal amount of electricity flowing from the electrodes to charge it. With it, polarization troubles were always either reduced to a constant factor or totally eliminated.

Switch (W_2) was wired to connect the quadrant electrometer either to the streaming potential cell or to a potentiometer (L) which served as a calibrating instrument. It was possible to read potentials to an accuracy of approximately one millivolt.

The conductivity of the liquid in the diaphragm was measured with the aid of a bridge (B), ear phones (A), and high resistances (Re).

The value of (λ_s) was determined by measuring the resistance across the diaphragm while the liquid, against which the ζ-potential is desired, was in the diaphragm pores. Then, after all streaming potential measurements were completed upon the sample, the "cell constant" of the diaphragm was obtained by replacing all liquid in the diaphragm with N/10 KCl (or a more concentrated soln., if needed), measuring the resistance across it and calculating the cell constant in the usual manner for any conductivity cell. Then from the measurements of resistance obtained while the experimental liquid was present, and the "cell constant," the specific conductivity (λ_s) of this liquid was calculated. Care was taken to use a standard solution of KCl (or other electrolyte) which was of sufficient strength to eliminate all surface conductance effects from the diaphragm, when it was present, in order that the value of the "cell constant" should be correct.

From the values of (P), (E), and (λ_s) thus obtained, ζ was calculated. The coefficient of viscosity (η) is equal to 0.01. (D), the dielectric constant of water, has the value 80. (P), which is observed in centimeters of mercury, must be expressed in absolute units, that is, in dynes. This is obtained by multiplying the cm. Hg. observed by the specific gravity of mercury, 13.6, and the force of gravity in dynes acting on one gram, i. e., 981. (E), observed in millivolts, must be divided by 1000 to reduce it to volts, and by 299.86 to reduce volts to c. g. s. electrostatic units. (1 c. g. s. electrostatic unit = 299.86 absolute volts.) (λ_s), observed in ohms^{-1} must be multiplied by 9×10^{11} to convert it into c. g. s. electrostatic units. (1 c. g. s. electrostatic unit = 9×10^{11} absolute ohm-cms.) The value of ζ obtained would be in electrostatic units. In order to obtain this value in volts, it must be multiplied by 299.86.

Then

$$\zeta = \frac{E\,\lambda_s}{P} \times \frac{9 \times 10^{11} \times 4 \times 3.1416 \times .01 \times 299.86}{13.6 \times 981 \times 10^3 \times 299.86 \times 80}$$

$$= 1.0596 \times 10^2 \times \frac{E\,\lambda s}{P}$$

where (ζ) is expressed in volts, (E) in millivolts, (λs) in reciprocal ohms, and (P) in centimeters of mercury.

Whether or not values of ζ obtained by the streaming potential method are identical with those obtained by the other methods of electrokinetic measurements is an important question which has had some attention but which needs more experimental work done on it. The electro-osmosis formula for the volume (V) flowing in unit time when the curent (i) is kept a constant, and the streaming potential formula given above are both independent of the dimensions of the diaphragm.

$$V = \frac{\zeta i\, D}{4\,\pi\,\eta\,\lambda} \quad \text{(electro-osmosis equation)}$$

$$E = \frac{\zeta\, P\, D}{4\,\pi\,\eta\,\lambda} \quad \text{(streaming potential equation)}$$

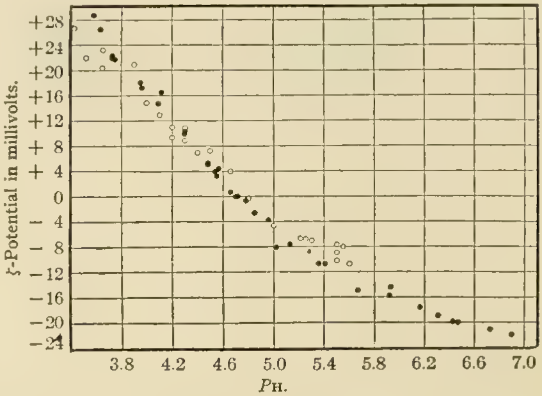
If these equations are correctly derived and no term is omitted from one which is considered in the other, the relation

$$V/i = E/P \text{ should hold.}$$

Saxen[18] determined with the same apparatus the electro-osmotically transferred volume (V), and also the potential (E), for the streaming current. He used a clay plate for the diaphragm and, as liquids, solutions of zinc, cadmium and copper sulfates with electrodes of the corresponding metals so as to eliminate polarization disturbances. The values which he obtained show a very close approximation to the identity of these ratios when the other variables are held constant. Values for the ζ-potential obtained by either the electro-osmosis, or streaming method, appear to be identical. Kanamaru[19], on the other hand, has recently measured the ζ-potential of cellulose and reports that $\zeta_S = 2.6\ \zeta_0$ where ζ_S = that obtained by the streaming potential method and ζ_0 = that obtained from electro-osmosis measurements.

Comparisons of values for ζ obtained from streaming potential measurements (ζ_S) and from electrophoresis measurements (ζ_E) have been made in the case of egg albumin. Abramson[20] made measurements of the electrophoretic velocity of quartz particles covered with egg albumin, suspended in 0.02 M acetic acid-sodium acetate buffers. Briggs[21] measured the streaming potential on a diaphragm of quartz powder, the surface of which was saturated with egg albumin. He used solutions which were .0004 M in HCl and LiCl, it being impossible to use the more concentrated buffer solutions in the streaming potential determination. While conditions were not identical in the two sets of experiments, Fig. 2 shows that the agreement of the calculated values of ζ were very close. The values for ζ_S in this case were slightly higher than those of ζ_E, this deviation becoming more marked at those pH's most distant from the isoelectric point of the protein. Abramson and Grossman[22] later made electrophoresis measurement on egg albumin covered particles of quartz, using buffer solutions identical with those employed in the streaming potential experiments. In this case, the values of ζ_E at those pH's distant from the isoelectric point were higher than those of ζ_S, although the difference obtained is small and is possibly due entirely to differences in the purity of the protein used in the two cases.

While more investigation is needed on this problem, it appears from existing data that values of ζ calculated from electrophoresis, electro-osmosis and streaming potential measurements are probably identical, which, of course, should be the case if the various equations are correctly derived.



●, By streaming potential method; ○, by Electrophoresis, measurements by Abramson.

Fig. 2.—The variation of the ζ-potential with PH on egg albumin.

Most measurements of the streaming potential have been made with glass capillary tubes. If the streaming potential equation is correct, the ratio E/P should be constant for a given solution and given tube throughout the entire pressure range wherein non-turbulent flow occurs through the tube. Also since the dimensions of the capillary do not appear in the equation, the ratio E/P should be independent of these dimensions so long as the tube (or diaphragm) consists of the same kind of material in all cases and the nature of the liquid phase is kept constant. In general these relationships have been found to hold true, but exceptions have been observed from time to time, which, in finally proven true, will require a revision or extension of the streaming potential equation.

Kruyt[23], and later Kruyt and van der Willigen[24], used the streaming potential to study the comparative effects of electrolytes on the ζ-potential of a surface and its relation to colloid stability. They used glass tubes of various lengths and areas of cross section, and streamed electrolytes of low concentration through them, under measured hydrostatic pressures. They measured the E. M. F. set up across the ends of the tube by means of non-polarizable Ag-AgCl electrodes and a potentiometer, using a capillary electrometer as the null instrument. For a given sample of glass and a given liquid, they found the value E/P to be constant, but to be different for different samples of glass. Kruyt points out that Grumbach[25] in some studies on the influence of non-electrolytes, in a millimolar KCl solution, upon the streaming potential, found that there was some change in the E/P value given, as the cell was allowed to stand for a few days. He verified this observation, but found it to be so small as to be negligible for all practical purposes. This shift

of the value of the ratio E/P with time seems to be a rather general observation and is evidently caused by some change in the chemical nature of the surface of the solid as a result of long static contact with the liquid. It probably indicates a real change in the ζ-potential and therefore does not detract from the accuracy of the streaming potential equation.

Briggs[15], studying the streaming potential across a diaphragm of cellulose pulp, found E/P to be constant for a diaphragm of given density. When, however, the density (i. e., the amount of pulp packed into a given volume) was changed, the value of E/P varied. This apparent deviation from the theory was found to be due to the fact that, in such a diaphragm, the conductivity of the liquid in the pores was greatly influenced (at low electrolyte contents) by surface conductance. The specific conductivity of the liquid in bulk (λ) therefore could not be considered constant and unchanged when it occupied the pores of the diaphragm, as is the case with glass capillaries. It was necessary to measure the specific conductivity of the liquid while in the diaphragm in the manner already described and use this value (λ_s) in the streaming potential equation in place of λ. The value

$$\frac{E\,\lambda_s}{P}$$

was found to be constant so long as the same sample of cellulose pulp was used against the same liquid.

Martin and Gortner[16] worked with cellulose diaphragms and used, besides water, many organic liquids as the fluid phase. They reported that the value

$$\frac{E\,\lambda_s}{P}$$

was not constant with pressure but increased with increased P until a constant value was obtained at fairly high pressures (above 200-400 mm. Hg.).

Bull and Gortner[26][27] found

$$\frac{E\,\lambda_s}{P}$$

to be constant for cellulose diaphragms but to vary with P for a powdered quartz diaphragm the particle size of which was not uniform. For diaphragms of uniform particle size,

$$\frac{E\,\lambda_s}{P}$$

was constant but varied from diaphragm to diaphragm according to the particle size, being lower for lower particle diameters. With particle sizes varying from 100 μ down to 4.5 μ this variation in

$$\frac{E\,\lambda_s}{P}$$

was most noticable. They conclude that the change in

$$\frac{E\,\lambda_s}{P}$$

with particle size is an evidence of an actual change in the ζ-potential with particle size and that the variation in

$$\frac{E\,\lambda_s}{P}$$

with P, when using a diaphragm of non-uniform particles, is due to some rearrangement in distribution of the particles in the path of the streaming liquid as a result of higher pressures. Since the phenomena is reversible, there would have to be a reversible change in the arrangement of the particles.

Similarly, White, Urban, Krick, and Van Atta[28] [29] have found that the H/P value for capillaries with diameters above 60 μ is constant but becomes progressively much lower as the diameters drop below this value. Whether this may be due to an unmeasured increase in surface conductance, to a decrease in ζ, or to a failure in the streaming potential equation to account for all the variables acting in cases of small pore diameters, it is not yet possible to decide. Bull[30] (see also, Abramson[45]) has offered a very plausible explanation of this phenomenon based upon the hypothesis that, in tubes of small radius, a counter pressure derived from electro-osmosis becomes important as compared to the applied pressure and thus gives rise to an apparent decrease in the E/P ratio, and in ζ, when in reality there is no change in ζ. The streaming potential, E, set up as the result of forcing a liquid thru the tube should be capable of acting electro-osmotically upon the liquid in the tube. The electro-osmotic pressure, P_1, would act counter to the hydrostatic pressure applied to the liquid, and could be defined by the equation

$$P_1 = \frac{2\,\zeta\,E\,D}{\pi\,r^2}\,.$$

The equation defining the pressure effective in setting up the streaming potential would be

$$P_2 = \frac{4\,\pi\,\eta\,\lambda\,E}{\zeta\,D}\,.$$

At equilibrium the applied hydrostatic pressure, P, would be partially neutralized by P_1, the elec-

tro-osmotic pressure, so that P_2, the effective pressure in determining the streaming potential, would be less than P by the amount P_1.

That is,

$$P = P_1 + P_2 = \frac{2\zeta E D}{\pi r^2} + \frac{4\pi\eta\lambda E}{\zeta D}.$$

The ratio P/P_1 would then be

$$P/P_1 = \frac{2(\pi r)^2\eta\lambda}{(\zeta D)^2} + 1 \cdot$$

From this equation it is seen that, as the radius of the tube becomes very small, P_1, becomes increasingly important as compared with P. From the data of White, Urban, Krick[28], and that of Bull and Gortner[27], Bull calculates that for tubes having a radius of 1 μ the value for P_1 is equal to about 1% of P while for tubes of 0.1 μ the value of P_1 is about equal to P_2, i. e., about 50% of P.

Ettisch and Zwanzig[31] have found an increase in the value of E/P (in a glass capillary) with increasing pressure up to a pressure of about 20 cm. H_2O, when aqueous solution of NaCl was the liquid being forced thru the tube. After this value of P was reached, the value of E/P became nearly constant with increased values of P. When alcohol-NaCl solutions were used as the streaming liquid the value of P above which E/P became constant was much higher. Reichardt[32] offers an explanation of these observations by picturing a difference between x, the distance apart of the double layers, and L, the distance from the wall at which maximum velocity of flow is attained and thru increments of which the viscosity is at variance (especially in immediate environment of wall) with the viscosity for the whole. Thus if $x < L$ and η signified the viscosity effective thru distance x, and η, that thru distance, L, then ζ would =

$$\int_0^x \eta/\eta' \, d\phi, \text{ not } \int_0^x d\phi = \phi_i - \phi_A,$$

as in Helmholtz's equation. By assuming that the boundary viscosity η' is a function of the applied pressure forcing the liquid thru the tube, which is in accord with the theory of Lamb[11], it is possible to explain the results of Ettisch and Zwanzig.

However, that such an explanation is needed has not been born out by results obtained by Bull[33] when repeating the work of Ettisch and Zwanzig. Bull used a pyrex tube and eliminated rubber connections in the system. He studied the streaming potential at pressures varying from 9 cm. H_2O to 90 cm. H_2O, and with mixtures of 10^{-5} normal NaCl and ethyl or isopropyl alcohol, in which the alcohol concentration varied from 0 to 80%. He finds there is no evidence of a change in ζ with pressure and in only two cases did the curve ,which is obtained by plotting E against P, fail to pass thru the origin. In all cases the curve was a straight line but in these two cases it failed to pass thru the origin. This was exactly what Ettisch and Zwanzig's data showed also, i. e., in their observations, E/P was not a constant but E/P + p was a constant, where p is the value of P when E = O. Bull found that in the cases of the two exceptions observed, when the experiment was repeated after the system had stood for several hours, the discrepancies disappeared and values of E/P became constant thruout the whole range of pressure. The curves of E against P plotted from data obtained before and after standing were parallel (i. e., their slopes were identical) the difference being that the former curve failed to pass thru the origin while the latter did so. Bull feels that such results as those of Ettisch and Zwanzig may be due to failure to allow sufficient time for equilibrium to be attained in the interface before the readings are made and that when this is done E/P for a given tube will always be constant.

From these data it seems probable that the streaming potential equation fails to hold for pore diameters which are very low. That it also fails for low values of P is not so likely . However, careful work is yet required to definitely settle these questions.

The streaming potential method for estimating ζ has been found useful in numerous cases.

Freundlich and Rona[34] and Freundlich and Ettisch[35] used this means to determine the value of ζ for a sample of glass for which they also measured the ϵ, or transverse, potential by use of the glass electrode of Haber and Klemensiewicz[36]. They found that ζ was not a function of ϵ and could vary entirely independently of it.

Kruyt[23] used this method to demonstrate the lyotropic and valence effects of salts upon the ζ-potential at a glass-water interface. Lachs and Biczyk[37] have repeated part of this work. Briggs[38] [39], using a cellulose diaphragm, studied the lyotropic and valence effects of various salts upon the ζ-potential and upon the surface conductance along the interface cellulose-water solution. He found, contrary to the theory of Smoluchowski[40] which postulates that there

should exist a direct relationship between these two interfacial phenomena, that there was no apparent relationship between them. This relationship has been further studied by Bull and Gortner[26].

Bull and Gortner[41] measured the temperature coefficient of ζ at water-cellulose and alcohol-cellulose interfaces thru the temperature range of 20°C.-51°C. by means of the streaming potential method. They confirmed the earlier findings of Cruse[41], who, working with a clay diaphragm, and measuring ζ by the electro-osmosis method, found a maximum in the temperature-ζ-potential curve for the water-solid interface at about 37°C-40°C. The temperature coefficient for the alcohol-cellulose interface was positive thruout the range of temperature studied. The same authors[43] demonstrated that no antagonistic action upon ζ exists between ions of Na and Ca, K and Na, or Ca and Mg at a cellulose-water interface, the effects of such ions being nearly additive in all cases.

Martin and Gortner[16] and Jensen and Gortner[17] have studied the streaming potentials set up when pure organic liquids were forced thru cellulose and Al_2O_3 membranes. The calculated values of ζ thereby obtained have been shown to bear an interesting and fundamental relationship to the molecular structures of these liquids. In a homologous series of alcohols, for instance, the value calculated for ζ varies regularly in a stepwise manner from one membrane of the series to another. That the streaming potentials obtained in such systems bear a definite relationship to the dipole moments of the molecules of the liquids being used, is indicated by the fact that those liquids which consist of symmetrical molecules, such as benzene and carbon tetrachloride, give no streaming-potential at all, while those which are known to have very high dipole moments, such as nitrobenzene, show the highest streaming potentials.

Bull and Gortner[44] have studied the streaming potential at a liquid-liquid interface. In these experiments a small stream of aqueous solution was forced from a jet containing one electrode thru a volume of white paraffin oil after which it impinged upon another electrode. They point out that while comparative values could be obtained for the streaming potential at the liquid-liquid interface in this manner, the values for ζ calculated therefrom on the basis of the streaming potential equation were not correct because of the fact that neither side of the interface was static. The term for slippage along the wall at the interface as introduced by Lamb[11] could certainly not be neglected in such a case.

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DISCUSSION

Dr. Mudd: Has it been your experience that all electrodes come to a constant value and remain there as long as the pressure is constant? In some work which I did they did not come to a constant value.

Dr. Briggs: Generally the values are quite reproducible. Polarization of the electrodes during the measurement must be prevented. Using a quadrant electrometer, the circuit is open at all times and a negligible amount of current flow is needed to charge the electrometer. There is, therefore, no appreciable amount of polarization, and the potential difference measurement remains constant as long as the pressure remains so.

Dr. Cohen: To what extent are the values reproducible?

Dr. Briggs: Using the same diaphragm and the same liquid the ratio E/P is constant. The value of E is read to an accuracy of about 1 millivolt, the percentage error in E is, therefore, higher as the value of E decreases. The value of E/P is then less accurate at low values of E than at high.

Dr. Fricke: Is it possible to obtain streaming potentials for tissues?

Dr. Briggs: By forcing a liquid of sufficiently low conductivity through a tissue membrane it should be possible to obtain a streaming potential. Surface conductance would be high, in all probability, and the accuracy with which Zeta could be calculated would depend upon how accurately this quantity could be measured.

Dr. Cole: I would think the difficulty there would be that of determining the cell constant of the tissue, because the size and shape of the pore enters implicitly though not explicitly. It is necessary to find out what the specific conductance of the liquid is when it is inside the pore. If one is trying to work with a live membrane, there would have to be doubt as to whether or not it was still alive throughout the whole course of the measurement.

Dr. Briggs: Streaming potential measurements can not be made on systems in which the specific conductivity of the liquid is high, as would be the case with most tissues bathed in their normal fluids. The chief difficulty of making streaming potential measurements on living tissues is this,—that the liquid phases of such systems are relatively concentrated electrolyte solutions.

Dr. Müller: What is the order of change in Zeta potential with temperature, in the cases investigated?

Dr. Briggs: For the cellulose-water interface the variation is within a range of 2 to 3 millivolts within the temperature range studied (20° C — 51° C). At the cellulose-alcohol interface the Zeta potential varied through a range of about 30 millivolts for the same temperature change.

Dr. Müller: This is quite in agreement with what one would find using the theory of the diffuse double layer. In this comparison of your measurements with the electrophoresis measurements of Zeta is the factor 4 or 6 used in the electrophoresis equation?

Dr. Abramson: The factor 4 was used. The evidence which yields a good deal of justification for using the same factor in both equations will be presented in my paper on The Chemical Constitution of Amphoteric Surfaces.

Dr. Fricke: To what extent could the deviation of E/P at low values of radius of the pore be accounted for by electro-osmosis?

Dr. Briggs: Bull has made measurements which thus far indicate that not all the deviation observed can be accounted for by the electro-osmosis counterpressure, i. e. that at low values of pore radius there is an actual falling off of the Zeta potential.

Dr. Cohen: Are these pores so small that the diffuse double layer would overlap in the center of the tube?

Dr. Briggs: The effect begins to be apparent in tubes having diameters less than 60μ.

Dr. Müller: It is hardly to be expected that the overlapping of the double layer would occur in tubes of this diameter although it might do so if the electrolyte concentration of the liquid were very low.

SURFACE CONDUCTANCE

Kenneth S. Cole

Smoluchowski Concept

The effect of a charged surface on the conductivity of an electrolyte in contact with it was first recognized by Smoluchowski in 1905. He postulated the "rigid," or Helmholtz, double layer with a surface charge density, $-\sigma$, for example, Figure 1, and an equal opposite charge σ at a distance d in the electrolyte. When an electric

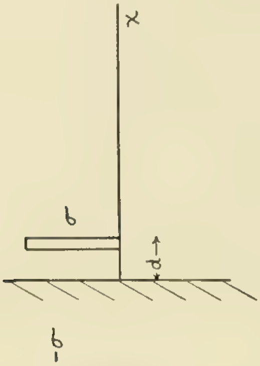

Figure I

field X is applied parallel to the surface, there is a uniform velocity gradient over the distance d, due to the force on the charge σ, and there is a uniform velocity for $x > d$. This is the phenomenon of electro-osmosis, and the velocity as given by Helmholtz is

$$u = \frac{X D \zeta}{4 \pi \eta} = \frac{X \sigma}{\eta} \cdot d \qquad (1)$$

where ζ is the potential difference across the double layer, D the dielectric constant, and η the viscosity. The charge σ is then moving with the velocity u and as such constitutes a current density per unit length of surface

$$J = X u \sigma$$

and the surface conductance

$$\lambda_1 = \frac{\sigma^2}{\eta} d = \frac{1}{\eta d}\left[\frac{D \zeta}{4 \pi}\right]^2 \qquad (2)$$

Diffuse Layer Concept

It is only recently that quantitative data on the phenomenon have been taken, but at the same time it was being generally realized that the Helmholtz double layer concept would have to be replaced by that of the diffuse ionic layer of Gouy, (1910), and Debye and Hückel, (1923),

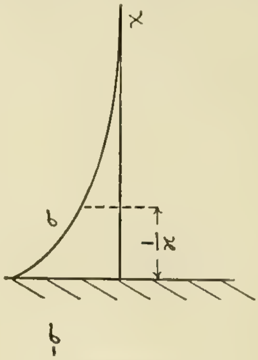

Figure II

Figure 2. Using the Debye approximation for low values of the ζ potential, the potential ϕ at a distance x from the surface is

$$\phi = \frac{4 \pi \sigma}{D \kappa} e^{-\kappa x},$$

where κ is probability thickness of the ion cloud. Then the charge density at x

$$\rho = -\frac{\kappa^2 D}{4 \pi} \phi = -\sigma \kappa e^{-\kappa x}.$$

Electro-osmosis takes place with the diffuse layer in exactly the same manner as for the Helmholtz layer except that the force of the field X on the part of the charge in a layer of thickness d x moves it a little faster than the adjacent layer nearer the surface and all of these increments of velocities have to be added together to give the velocity at any distance x. The force equation

$$F = -\eta \frac{d^2 u}{d x^2} = X \rho = -\frac{X D}{4 \pi} \cdot \frac{d^2 \phi}{d x^2} \qquad (3)$$

when integrated gives,

$$u = \frac{X D}{4 \pi \eta}(\phi - \zeta) = -\frac{X \sigma}{\kappa \eta}\left(1 - e^{-\kappa x}\right) \qquad (4)$$

The current density due to the charge carried is then

$$J = \int_0^\infty u \rho \, d x = \frac{X \sigma^2}{\eta} \int_0^\infty e^{-\kappa x} (1 - e^{-\kappa x}) \, d x$$

and the conductance,

$$\Lambda_1 = \frac{\sigma^2}{2 \kappa \eta} \qquad (5)$$

Thus we see that the consideration of a diffuse layer has not changed the surface conductance equation derived on the basis of a Helmholtz layer when d is replaced by ½ κ.

Ionic Mobility Concept

There is, however, another factor, unknown to Helmholtz and not considered by Smoluchowski, that becomes obvious from our present knowledge of the structure of the diffuse layer. We have said, until the present, that the electro-osmosis arose from the force exerted by the electric field on the net charge in the water without considering the manner in which this force is transmitted to the water. When the field X is applied, it exerts a force on each ion of $X z_i \mathbf{e}$ (where $\mathbf{e}$ is the electronic charge and z_i the valence) and in a very short time the ion reaches a velocity where the viscous friction of the water prevents a further increase and the velocity becomes constant. The reaction is then a force $X z_i \mathbf{e}$ that is applied by each ion to the water by its motion through the water. If there are equal numbers of the two ions of opposite charge, then the forces due to each are equal and opposite and there is no net force on the water. If there is an excess of positive ions, they will exert more force on the water than the smaller number of negative ions which oppose the motion, and the water will tend to move with the positive ions, but the velocities of ions of both signs, relative to the water in their immediate vicinity, will remain unchanged in spite of whatever movement there may be of that water as a whole. This motion of the ions through the water is not of primary importance in other electrokinetic phenomena but should usually be responsible for a considerable proportion of the surface conductance. Electrolytic conductivity depends upon the concentration of ions present, the velocity with which they move, and the charge they carry. If the measurement is made at a great distance from all boundaries, there are equal positive and negative ionic charges and the water does not move as a whole, so we have the normal or bulk conductance. If the presence of a charged surface alters the velocity or the concentration of any ions, then there is a change of conductance in that region. The difference between the bulk conductance and the observed conductance is then the surface conductance. Thus if a surface repelled ions of both signs (as at an air-water interface), the concentration of ions would be less, the conductance *less* than the bulk conductance, and this particular surface conductance would be negative. In a similar manner, if the concentration of one ion is decreased from normal, the sur-

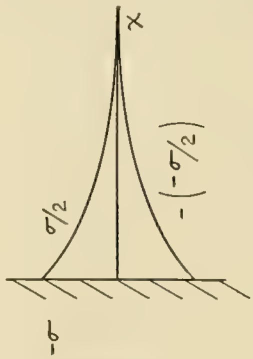

Figure III

face conductance due to it is negative. But if, at the same time, the concentration of another ion is increased above normal, Figure 3, the surface conductance due to it will be positive, and the effect of both ions, depending upon which predominates, may be positive, negative or zero.

General Formulation

In order to use these ideas we shall express the total conductance at any arbitrary point in the electrolyte. As has been said, the conductivity may, to a first approximation, be taken as the sum of the conductances due to the separate ions. The current density is then $J_i = X u_i n_i z_i \mathbf{e}$ where u_i is the total velocity of the i ion under unit field, n_i and z_i are the concentration and valence respectively. Then the total current density

$$J = \Sigma J_i = X \Sigma u_i n_i z_i \mathbf{e}$$

and the conductance

$$\Lambda = \Sigma u_i n_i z_i \mathbf{e} \qquad (6)$$

The velocity u_i is made up of velocity of the ion relative to water, which is the usual mobility u_i plus the velocity of the water u. If we further say that the molecular concentration of salt in the center of a large body of electrolyte is n molecules per cc. and each molecule is completely dis-

sociated to produce ν_i ions of i type, and at the point under consideration there is a fractional change of the concentration of the i ion Δ_i, we have

$$\Lambda = \Sigma\, (u + u_i)\, n\, \nu_i\, (1 + \Delta_i)\, z_i\, \mathbf{e} \qquad (7)$$

Separating

$$\Lambda = u\, n\, \mathbf{e}\, \Sigma\, \nu_i\, z_i + n\, \mathbf{e}\, \Sigma\, u_i\, \nu_i\, z_i$$

$$+ u\, n\, \mathbf{e}\, \Sigma\, \Delta_i\, \nu_i\, z_i + n\, \mathbf{e}\, \Sigma\, \Delta_i\, u_i\, \nu_i\, z_i$$

Since there is electric neutrality in the bulk of the liquid $\Sigma\, \nu_i\, z_i = 0$, while $n\, \mathbf{e}\, \Sigma\, u_i\, \nu_i\, z_i = \Lambda_e$ is the bulk conductance. The difference in conductance, which is the surface conductance, becomes

$$\Lambda_{\underset{0}{s}} = \Lambda - \Lambda_e = u\, n\, \mathbf{e}\, \Sigma\, \Delta_i\, \nu_i\, z_i + n\, \mathbf{e}\, \Sigma\, \Delta_i\, u_i\, \nu_i\, z_i \qquad (8)$$

Noting that $n\Delta_i\, \nu_i\, z_i\, e = \rho_i$ is the contribution of the i ion to the net charge density ρ

$$\Lambda_{\underset{0}{s}} = u\, \rho + \Sigma\, u_i\, \rho_i \qquad (9)$$

u, ρ and ρ_i are functions of the distance from the surface, so we must integrate to get the surface conductance

$$\Lambda_{\underset{0}{s}} = \Lambda_1 + \Lambda_2 = \int_0^\infty u\, \rho\, d\, x + \Sigma\, u_i \int_0^\infty \rho_i\, d\, x$$

If we let

$$\sigma_i = \int_0^\infty \rho_i\, d\, x$$

since it is the contribution of the i ion to the total charge

$$\Lambda_{\underset{0}{s}} = \int_0^\infty u\, \rho\, d\, x + \Sigma\, u_i\, \sigma_i \qquad (10)$$

Debye Approximation Calculation

In computing the diffuse layer analogue of the Smoluchowski conductance, we have already evaluated the first term with the Debye approximation. This approximation for a salt which ionizes into only two ions per molecule gives

$$\sigma_1 = -\sigma_2 = \sigma/2$$

so

$$\Lambda_{\underset{0}{s}} = \frac{\sigma^2}{2\, \kappa\, \eta} + \frac{\sigma}{2}\, (u_1 - u_2) \qquad (11)$$

For other salt types,

$$\sigma_i = -\frac{\nu_i\, z_i^2}{\Sigma\, \nu_i\, z_i^2}\, \sigma$$

and if we let

$$u_i = \frac{\mathbf{e}\, z_i}{f_i}$$

where f_i is the friction coefficient of the ion then

$$\Lambda_{\underset{0}{s}} = \frac{\sigma^2}{2\, \kappa\, \eta} + \sigma\, \mathbf{e}\, \frac{\Sigma\, \nu_i\, z_i^3/f_i}{\Sigma\, \nu_i\, z_i^2} \qquad (12)$$

This formula, which has been derived on the Debye approximation, is applicable to ζ potentials up to about 10 mv.

General Calculation

In cases where ζ is larger it is necessary to use a more exact formula, which is somewhat more difficult to develop. In order to show how this is accomplished, let

$$q = \int_x^\infty \rho\, d\, x = \frac{D}{4\, \pi}\, \frac{d\, \phi}{d\, x} = \sqrt{\frac{D\, k\, T\, n}{2\, \pi}}\, \sqrt{\Sigma\, \nu_i\, \left(e^{-\frac{z_i\, \mathbf{e}\, \phi}{k\, T}} - 1\right)}$$

Integrating by parts,

$$\Lambda_1 = \int_0^\infty u\, \rho\, d\, x = \int_{q_0}^{0} u\, d\, q = u\, q\Big]_{q_0}^{0} - \int_{q_0}^{0} q\, d\, u = -\int_{q_0}^{0} q\, d\, u \qquad (14)$$

Substituting for q, and noting

$$u = \frac{D}{4\, \pi\, \eta}\, (\phi - \zeta)$$

from (3) we have $\Lambda_1 =$

$$-\frac{D}{4\, \pi\, \eta}\sqrt{\frac{D\, k\, T\, n}{2\, \pi}} \int_\zeta^0 \sqrt{\Sigma\, \nu_i\, \left(e^{\frac{-z_i\, \mathbf{e}\, \phi}{k\, T}} - 1\right)}\, d\, \phi \qquad (15)$$

where ζ is the potential at $x = 0$ or $\phi_0 = \zeta$

If $v_1 = v_2 = v$, $\Lambda_1 =$

$$\frac{D}{4\pi\eta}\sqrt{\frac{DkTnv}{2\pi}}\int_0^{\zeta}\left(e^{\frac{ze\phi}{2kT}} - e^{-\frac{ze\phi}{2kT}}\right)d\phi$$

$$= \frac{DkT}{2\pi\eta e z}\sqrt{\frac{DkTnv}{2\pi}}\left(e^{\frac{ze\zeta}{2kT}} + e^{-\frac{ze\zeta}{2kT}} - 2\right) \quad (16)$$

For Λ_2 we must evaluate

$$\sigma_i = \int_0^{\infty} \rho_i \, dx$$

$$\sigma_i = n\,e\,v_i\,z_i\int_0^{\infty}\left(e^{\frac{-z_i e\phi}{kT}} - 1\right)dx$$

$$= n\,e\,v_i\,z_i\,\frac{D}{4\pi}\sqrt{\frac{2\pi}{DkTn}}\int_{\zeta}^{0}\frac{e^{\frac{-z_i e\phi}{kT}} - 1}{\sqrt{\Sigma v_i\left(e^{\frac{-z_i e\phi}{kT}} - 1\right)}}\,d\phi\,. \quad (17)$$

If $v_1 = v_2 = v$

$$\sigma_i = \sqrt{\frac{DkTnv}{2\pi}}\left(e^{\frac{-z_i e\zeta}{2kT}} - 1\right)$$

So

$$\Lambda_{s_0} = \frac{DkT}{2\pi\eta z e}(\sigma_1 + \sigma_2) + u_1\sigma_1 + u_2\sigma_2 \quad (18)$$

This expression can be consolidated considerably since

$$\sigma_i = \frac{\sigma}{1 + e^{\frac{-z_i e\zeta}{2kT}}} \quad ;$$

$$\sigma = \sqrt{\frac{DkTnv}{2\pi}}\left[e^{\frac{ze\zeta}{2kT}} - e^{-\frac{ze\zeta}{2kT}}\right] \quad (19)$$

$$\sigma_1 - \sigma_2 = \sigma \quad ;$$

$$\sigma_1 + \sigma_2 = \sqrt{\frac{\pi}{2DkTnv}}\left(\frac{\sigma}{\cosh\frac{ze\zeta}{2kT}}\right)^2,$$

and then

$$\Lambda_{s_0} = \frac{1}{2\kappa\eta}\left[1 + \frac{\pi\eta\Lambda_e}{DkT}\right]\left(\frac{\sigma}{\cosh\frac{ze\zeta}{2kT}}\right)^2 + \sigma e z\left(\frac{1}{f_1} - \frac{1}{f_2}\right) \quad (20)$$

Comparison with Other Derivations

The relation above is, of course, the same as that of Smoluchowski when $d = \frac{1}{2}\kappa$, the ionic mobilities are neglected and ζ is small, as has already been shown. The next development is that of Komagata, (1929), for the case of a circular pore when ζ is small enough for the Debye approximation to be used. The Smoluchowski conductance is neglected, and when κR ($R \equiv$ pore radius) is large, the Bessel function of the solution may be approximated to give the ionic mobility term of equation (11). The other extreme, where the thickness of the ion atmosphere is large compared to the pore radius, has been independently derived and will be discussed elsewhere. It is difficult to appraise the formulation by Mooney, (1932), since it differs from the above equation (18) and the derivation has not been available. With the exception of a single term, agreement is obtained if σ_1, σ_2 and σ have the coefficients

$$\frac{\omega}{1+\omega}, \ \frac{\omega}{1-\omega} \text{ and } \frac{\omega}{1-\omega^2} \text{ where } \omega = \sinh\frac{ze\zeta}{2kT}.$$

Mooney states that he has considered the distortion of the diffuse ionic layer by the electric field, and since this effect has not been considered here, it may well be the reason for the differences in the final result. The next derivation published was for a plane with small ζ, and both types of conductance were considered, giving equation (12), Cole, 1932.

It is difficult to discuss adequately the theory of Urban and White, (1932). They have assumed from Stern's theory, (1924), of double layer, that there is a number of cations equal to

$$\sqrt{\frac{DkTn}{2\pi \mathbf{e}^2}}\; e^{\frac{z\mathbf{e}\zeta}{2kT}}$$

near the surface and an equal number of anions on the surface, both of which give rise to surface conductance by moving with their respective mobilities as found in free solution. The reasons for splitting the equation (19) for σ and for taking a normal mobility for an adsorbed ion seem insufficient in view of the omission of the Smoluchowski term.

The recent work of Bikerman, (1933), seems accurate and complete, although the development is somewhat awkward due to the entire dependence upon the work of Gouy. The results are stated for a plane parallel slit, so the absolute values are for two square cm. of surface and therefore twice the above. The effect of the separation of the surfaces is considered in some detail and has, of course, been neglected here. Bikerman also makes the most interesting suggestion that the Smoluchowski term should drop out at a comparatively low frequency of measuring current while the ionic term should remain constant over the range of practical frequencies.

Experimental Results

After Smoluchowski's theoretical prediction of surface conductance, it was first found experimentally by Stock, (1912). There have been other qualitative observations of it which are quoted by Briggs, (1928), but the value of ζ has usually not been given so that it was not possible to make a quantitative check. The first satisfactory data that have been found are those of Briggs, (1928), on cellulose, and it is unfortunate that the surface area is not available for a determination of the absolute value of the conductance. Abramson, (1932), showed that for these data there was a qualitative agreement with Smoluchowski's theory when the ionic strength of the solutions was corrected for the ionic strength of water. Using these same data for the univalent chlorides it was shown, (Cole, 1932), that the conductance was a linear function of the surface charge, except at low surface charges, so that the ionic term was of much greater significance than the σ^2 or Smoluchowski term. It was necessary, however, to ascribe an added conductance to Cl^- and the question was left open as to whether this was due to a mobility of the adsorbed ions or due to the presence of molecular or Van der Waal forces which increased the concentration of ions of both signs near the surface in proportion to the charge.

There has been considerable further work on packed cellulose done in Gortner's laboratory, Bull and Gortner (1931), but it will not be considered here, since, as in Briggs' (1928) work, both the surface area and the pore diameters involved cannot be determined.

The first attempt at the absolute value of the conductance was made by McBain and Peaker, (1929), but the ζ potential is not given, and White et al, (1932), have not been able to reproduce the data. The conductance was considerably larger than calculated by Mooney from his formula, and he postulated a movement of adsorbed ions, (1932).

Urban and White, (1932), have given data for two concentrations of KCl in pyrex capillaries, but state in an earlier paper, White, Urban and Van Atta (1932), that the potentials are the maximum observed with freshly prepared capillaries and that ζ might decrease to the neighborhood of zero with but slight effect on the conductance. However, the data are probably the best available, and a recomputation is worthwhile,

TABLE 1.

CALCULATION OF SURFACE CONDUCTANCE FROM DATA OF URBAN AND WHITE

KCl	ζ	σ	Λ_1	Λ_2	Λ_{So}	Λ_{So} obs.
$2.5 \cdot 10^{-4}$	120	2910	.58	.62	1.20	1.35
$5.0 \cdot 10^{-4}$	124	4650	.92	.99	1.91	2.24
M	mv.	$\frac{\text{esu}}{\text{cm.}^{-2}}$	$\times 10^9$ mho.			

(Table I). The agreement is not as good as that obtained by the authors with their formula. It is, however, in line with the conclusions drawn from the data of Briggs, and agreement is obtained if it is assumed that the charge adsorbed is only Cl^-, all of which moves with a velocity between 25 per cent. and 30 per cent. of normal. The weight of experimental evidence which is promised will decide whether Urban and White,

and Komagata or Smoluchowski, Mooney, Cole and Bikerman have a more nearly correct picture of the mechanism.

Remarks

The phenomenon of surface conductance has an apparently satisfactory and complete mathematical background that has had a rapid recent development. There is, at present, so little complete quantitative data that it is not possible to claim that the theory is well verified by experiment. The indications are, however, that the conductance, due to the changes of ionic concentrations near the charged surface, is of the same order of magnitude as the electro-osmotic (Smoluchowski) term in many cases, and that it may be necessary to supply a mechanism which allows ions of the sign of the adsorbed ions to play a more prominent role than the present theory allows. There will probably be need for an extension of the theory in the direction of smaller pores which are more often encountered in biological systems. It is to be hoped that the investigation of surface conductance will give us a clearer insight into the mechanism of interfacial adsorption and will help to formulate possible membrane structures that will perform biological tasks.

Discussion

Dr. Fricke: Would you care to discuss the justification for omitting the conductivity within the rigidity boundary?

Dr. Cole: It seems quite possible that the ions inside of the rigidity boundary may have a definite mobility in a tangential electric field, but, since this mobility is not known, it can only be included in the theory in the form of an arbitrary constant.

Dr. Müller: Is it correct to assume that the electric field is the same everywhere?

Dr. Cole: This assumption seems reasonable for the case of an infinite plane, and a circular pore, when the solid is an insulator.

Dr. Fricke: Serious errors might be committed when the pores are not straight and when end phenomena are present. Is one justified in assuming that the viscosity is the same in the diffuse layer as in the bulk of the liquid?

Dr. Abramson: Janet Daniels has measured the electrophoretic mobility of protein in a series of alcohol-water mixtures up to 35% alcohol, where the charge density was constant. The dielectric constant changed from 80 to 68 while the bulk viscosity changed .01 to .02. The dielectric constant enters as the square root, and the viscosity directly, so that the changes were largely due to the latter. The agreement of the observed and computed mobilities indicates that the viscosity in the diffuse layer is the same as in bulk.

What is found when the theory is arbitrarily made to agree with Briggs for KCl?

Dr. Cole: The formula will agree fairly well with the data on the univalent chlorides if it be assumed that all of the surface charge is due to Cl and that it can move with about one-third of the mobility in free solution.

Dr. Cohen: The introduction of this arbitrary correction factor, which causes the above data to fall into a consistent series, is but a single instance, and may be unique, therefore it does not merit undue importance. However, an examination of this "arbitrary" correction may furnish a hint toward the solution of the problem. It is conceivable that the behavior of the ions involved in surface conductance may be modified by the forces peculiar to surfaces in such a manner that ordinary concepts of concentration, etc., do not apply without correction.

Dr. Cole: One might expect the Smoluchowski effect to play a larger part than it does in Dr. Brigg's measurements. This might be an example for Bikerman's suggestion of the effect of the measuring frequency.

Dr. Fricke: Is there a possibility that part of the observed resistance may be derived from a polarization resistance in the cellulose diaphragm? If this were the case the resistance would depend on the frequency, and there should also be a capacity effect. A condenser would then be necessary to obtain a good bridge balance.

Dr. Briggs: Measurements were made at the frequency of one thousand cycles. A condenser was not used in bridge but the end-point was perfectly satisfactory except at low concentrations of KCl.

Dr. Mudd: I would like to suggest that there are a number of physiological problems that might conceivably find a solution in the application of this type of phenomena. There are the questions of the kidney and intestine secretion against osmotic pressure which are not explained by the ordinary laws of diffusion (and osmotic pressure.

Dr. Abramson: Has the surface conductance at protein surfaces been measured?

Dr. Briggs: In my measurements of the streaming potentials of protein the surface conductance was so low that it was practically negligible. Bull has also found this to be the case.

Dr. Cohen: It seems clear that experiment lags far behind theory in the study of surface conductance. Moreover, Dr. Cole's formulation

of the theory shows that surface conductance on a solid in contact with an electrolyte is beset with serious complicating factors that cannot be resolved readily, if at all. The latter appear to be absent in the case of surface films between a solvent and its vapor phase, or an inert gas. Since films with measurable dimensions can be prepared from a variety of solvents and solutes, it would seem possible to obtain experimental data of sufficient variety to form a basis for test of the theoretical aspects.

Dr. Cole: I think that Dr. Cohen's suggestion is excellent. The conductivity vs. thickness data for films and ζ potential data from electrophoresis of air bubbles should allow calculation of the surface conductance and indicate the faults in the theory.

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ON THE ABILITY OF MAMMALS TO SURVIVE WITHOUT BREATHING

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The subject about which I wish to speak tonight is the ability of warm-blooded animals to survive under circumstances in which respiration is temporarily impossible. In the case of man, the requirement of oxygen is one of the most insistent and will not tolerate any interruption for more than a minute or so. It is quite different from the other essential requirements. The period during which an individual may survive without respiration, being limited to such a short time, leaves only a very narrow margin separating him from the termination of existence from lack of oxygen.

This narrow margin has had an extremely important influence on the habits and activities of human individuals. The termination of human life usually results from the interruption of respiratory activity as a whole. Or it may be that some essential link in the respiratory or circulatory system is affected and the organism ceases to function. The peril of death is always close in the matter of respiratory function, and realization of the imminent danger of asphyxia brings forth the most powerful protective responses.

The period during which one may reasonably hold his breath determines the timing of musical notation. It restricts the human environment and sets a definite limit as to time and depth to which man may penetrate the sea.

If the respiration were temporarily arrested, the period to which any one of us could hold his breath would be about forty-five seconds. If, however, we made some previous preparation, such as forced deep breathing, that period might be extended to five or six minutes. If to that period of preparatory deep breathing, were added the respiration of oxygen, the time might be extended to 10-15 minutes. This device was made use of in the preparation of sprint swimmers from one country in the last Olympic games.

The rate of oxygen consumption is probably the factor which determines the time during which the breath may be held. For a person at rest we can take the oxygen consumption to be 250 cc. per minute; with only quite moderate activity, such as walking, 7-800 cc. per minute is required. For climbing or moderately vigorous exercise, the consumption may rise to 1200 cc. per minute. A Marathon runner, running seventeen kilometers per hour may consume as much as 3500 cc. per minute. Against these requirements we find that the ordinary individual has a certain capacity for the storage of oxygen. Storage in the lungs is one important factor to consider. The so-called "vital capacity" amounts to about 10% of the body weight. A 70 kilogram man would thus contain 7 liters of air in his lungs. Of the seven liters of air, less than one-fifth would be oxygen available for respiratory requirements. The quantity of oxygen which would be present in the lungs would then not amount to more than 1200 ccs. Furthermore, a certain amount of oxygen which would be stored in the blood would be available. Allowing approximately 7% of body weight as made up of blood, with an average oxygen content of 16-17

ccms. per 100 ccm. of blood, we should find that the total quantity of oxygen in the blood would be 800 ccs.

In addition to these more apparent stores of oxygen, we have other body fluids which are more or less saturated with oxygen. Assuming the tension of oxygen in tissue fluids to be about equal to atmospheric oxygen, there could be dissolved in the tissues 1000 ccs., making the total amount stored in the human body about three liters. This, of course, is a maximum figure. Actually it is quite impossible that the entire quantity would be available for use; there must always exist a certain gradient which serves to force the oxygen from the places where it is stored into the muscles and other tissues where it is to be consumed. Probably not more than one-half the total stored oxygen would be available for use if respiration were arrested. This would amount to about 1500 ccs. Since the oxygen requirement at rest is 260 ccs. per minute, this would seem to allow for survival without respiration for about 6 minutes. That is just about the limit of human survival without previous preparation, but it is not possible to hold out so long voluntarily.

It is also true that in addition to the store of oxygen that is dissolved in tissues and blood there exists in the "oxygen debt" an important means for maintaining the muscle tissues during a period when oxygen is not available. It operates by the transformation of glycogen into lactic acid without the intervention of oxygen. This reaction yields energy which can be applied to muscular activity. When oxygen is available, the lactic acid is removed by an oxidative process. The oxygen debt can be measured by determining an individual's basal metabolic requirements, getting him to engage in violent muscular activity and measuring the oxygen consumption during recovery. More oxygen is invariably consumed during recovery than is needed for ordinary maintainance and this extra amount indicates to what extent the individual has gone beyond his means during the activity. A supply of energy becomes available in this way which would be equivalent to that furnished by fifteen liters of oxygen in the case of a trained athlete.

In contrast to the total stored oxygen of three liters, fifteen liters is quite a large amount, and one would be inclined to turn to it to provide means for survival when respiration is arrested. However, it is available only for muscular activity, and is not used by any tissue other than muscle. So that while the oxygen debt may serve very well to carry on muscular activity during a short period of asphyxia, it does not solve the problem for the important non-muscular tissues, such as the heart and brain.

Since the resources which have been examined do not provide the means for survival during extended periods of asphyxia, we can scarcely see how it would be possible for man to adapt them to make longer survival possible. Many animals, on the other hand, are superior to man in this respect. The first systemic work on this subject was done seventy years ago by Paul Bert. He determined the period during which a number of animals survived forced submergence under water. Various terrestrial animals, such as the dog, cat, rabbit, and hen, survived for from 2 to 4 minutes under these conditions. Similar tests of animals with a partly aquatic habitat, such as gulls, indicated that they were not particularly superior to the others in this connection. But the ordinary domestic duck, which is usually not an actively aquatic animal, survived for a period of 10-15 minutes; a seal, (one which was not in very good condition) was still moving at the end of 15 minutes, and the heart continued to beat for 28 minutes after immersion.

It is of interest to consider his observations on the peculiar ability of young animals to resist asphyxia. New born rats would survive thirty minutes; for adults the limit of survival was about two minutes. During development the period which they survived progressively diminished. This remarkable capacity for resistance to asphyxia in new born animals is probably related to the necessity for sudden development at birth from a condition in which the respiratory apparatus has not been in use. At the time of birth in mammals there must occur a period when respiration as such is quite impossible. Respiration starts somewhat hesitantly and only after some time does it become regularly and firmly established, but once established there is no further interruption.

The above experiments would appear to be rather artificial, and would scarcely give the animal a chance to demonstrate its full ability to resist asphyxiation. On looking through the literature to ascertain the opinion of the various authors on the duration of the period under which diving animals can survive, we find that the information is quite uncertain. Hill has said that the human limit was probably that of a pearl diver, who remained submerged for over 4 minutes. Parker mentions that the regular respiratory interval of the Florida manatee may be as long as 20 minutes. Beyond this the figures are not so certain.

The largest mammal, which is most able in its capacity to dive, is the whale. On account of the conditions of observation and the somewhat romantic flavor which attaches itself to whaling stories, we find that authors show a good deal of hesitation in committing themselves as to the

period during which a whale can remain submerged. This hesitation is unfortunately not apparent with other authors—one reference states that a whale may remain submerged for as long as 10-12 hours. Most of the publications which one would consider fairly reliable, however, set the limit of submergence at about an hour or an hour and a quarter. It is difficult to make accurate observations of this kind. I tried myself one time to time the dive of a loon. The loon submerged and the next time I saw him was about 12 minutes later. Some people, and I am among them, are skeptical of this measurement, and others think it unfair—that the loon could actually do much better than that. There are reports that loons have been caught on set lines at 150 ft. depth, which shows its ability to travel under water. The "Old Squaw" is said to have been captured in nets in the Great Lakes at depths of 180 ft., which indicates a considerable diving ability.

So we see that certain mammals have a capacity for resisting asphyxia which far exceeds the ability of man, and, as a matter of fact, exceeds the capacity which we would expect on the basis of the amount of oxygen stored. That being the case then, we might consider what possible modifications might occur which would adapt them to survival. There might be an increased vital capacity, with an increase of air stored in the lungs. This capacity might possibly be doubled; on the other hand, if it were to be much more than doubled there would hardly be space available in the body to accomodate it. Birds in particular have made use of the larger capacity of their respiratory apparatus to increase to some extent their capacity for survival. Mr. Foster and I examined the vital capacity of the duck, and found that its vital capacity was actually double that which would be expected from a mammal of the same size. But this increase is not sufficient to account for the enlarged capacity for survival which has been observed in certain animals.

An increase in the volume of blood might be considered as a possibility. So many authors have called attention to the vascular networks of many diving animals, the so-called retia miriabilia which are quite conspicuous and which would seem to give to the animal a greater capacity for blood and hence for oxygen storage in the blood. It is very difficult to see, however, where there would be space for the visceral and other organs if the vital capacity were to be doubled, and the blood content also. Doubling the vital capacity and blood volume would take up 35% of the entire body weight. If then we could give the animal only a slight addition to its ability to survive, making the limit three minutes, possibly, instead of 1-2, it would not seem worth while to consider these modifications in the respiratory or circulatory system as sufficient to adapt an animal like the whale to attain its outstanding submergence and under water activity.

We should also consider the capacity for contracting a greater oxygen debt. That seems at first sight a tempting possibility to investigate. But if we were to increase the oxygen debt, it would only influence the capacity for maintaining muscular activity for a longer period of time. I do not think, therefore, that the difficulties of non-diving animals rests in the maintenance of the muscles; the oxygen supply for an arm or leg may be cut off for 10-15 minutes without great discomfort, and for an hour without actual serious damage to the part. The difficulty seems to arise in protecting the more sensitive tissues, such as the heart and brain, which are damaged at once by asphyxia.

It usually happens that when we examine an animal part by part and then attempt to recreate the whole by the addition of parts, we find that the whole animal is quite different from the sum of its component parts. There are additional processes by which parts are made to cooperate together, a particular type of integration which completes the working organism.

The mechanism for the control of this integration we regard as the central nervous system. If we belong to one group of biologists, we are likely to call it behavior, or, examing it as physiologists, we are more apt to analyze this behavior into its component parts, and refer to these as reflexes. It seems possible that it might be pertinent to examine some of the reflexes of diving animals to see whether they may be responsible for some degree of protection from asphyxia. The first information which appears was published again by Paul Bert on the behavior of the duck when forcibly submerged in water. It remained quite quiet for from 10 to 15 minutes. Just before the termination of its existence, it gave some convulsive movements, but remained otherwise very still. This is quite in contrast to terrestrial animals, which, when submerged, or when the trachea is clamped, immediately perform convulsive movements which blindly attempt to bring relief from asphyxia. They are quite purposeless movements which actually serve to terminate their existence more quickly. The duck, on the other hand, conserves its energy by eliminating all muscular activity. That process has been quite definitely worked out as a particular reflex reaction, evoked by postural stimuli which give this reaction even when the animal is

out of water. The stimulus is given by holding the head or neck in a certain position, which is similar to the one assumed swimming under water, with the head and neck extended and slightly depressed. When a duck is held in this position, all activity of the animal ceases even though it is out of water. The same sort of reflex inhibition of muscular activity can also be seen in the muskrat under a stimulus of the same kind. Activity of the respiratory muscles is inhibited as well, respiratory movements are at once abandoned; a duck or muskrat can be held in that position for 10-15 minutes and no attempt to breathe will be made. That type of reflex is obviously of adaptive nature in favoring certain animals for existence under water.

Along with the depression of skeptical activity and the cessation of the working of the respiratory system, the following observation has been made by Richet. Inhibition and retardation of the heart beat occurred during submergence. When the vagus nerve in a duck is cut it does not survive any longer than a hen would under the same conditions. This reflex mechanism then seems to have a definite positive effect in protecting an animal during diving.

Even so, while it may inhibit the activity of the animal, it would not abolish its basal metabolic requirements, and we are still faced with the problem of how these animals survive for so long a period. Gratiolet in 1860 made certain observations on the vascular structure of the hippopotamus. He claimed that there was a muscular band which passed about the vena cava about where it went through the diaphragm. He believed it had the ability to contract and to prevent the remainder of the blood from returning to the heart. He thought that such a mechanism would be useful in preventing the engorgement of heart and brain which was supposed to occur during asphyxia. The idea of "engorgement" may be injected into the discussion by reason of one's own sensations during asphyxiation, when the cerebral vessels seem to be engorged and the heart feels strained; but whether the mechanism works in just that way seems rather doubtful. On the other hand, if such a device shut off a large part of the posterior returning circulation and shut it down to just what was able to pass through the anterior venous return then it would be possible for such oxygen as was stored to be utilized by those organs which are particularly sensitive to oxygen want. Other tissues can get along with the help of the oxygen debt process, but the heart and brain have no such device to aid them.

In recent years more and more attention has been paid to cerebral and coronary circulation, with the result that it is apparent that the condition of control in those systems is quite unique from the type of control extended over ordinary systemic circulation. We find suggestions of this in the apparently opposite action of histamine and adrenalin on coronary circulation. We learn also from Lenox and Gibbs, that if 5% carbon dioxide is breathed and the cerebral circulation is judged by the amount of oxygen in the blood returning through the jugular vein, that the cerebral circulation will be accelerated by as much as 40%, while circulation returning through the femoral vein is even diminished. There is then a relation between the cerebral and systemic circulation, which under conditions os asphyxiation would serve to conserve the oxygen supply for the more sensitive tissues, leaving the others to develop an oxygen debt.

I feel that when we cannot find either chemical or physical processes in the avian or mammal body which would adapt them for submergence, that we should turn our attention to reflex adjustments. It might be pointed out that the physical-chemical and muscular sysems in mammals are remarkably alike, for the blood and muscles apparently have the same characterists in all forms and the elements of the nervous system are so similar as to be practically identical. So we can see that throughout the operation of the forces of evolution the physical and chemical processes have remained extremely constant, maintained in an apparently rigid mold. On the other hand, the adaptation of various groups to different environments indicates a remarkable degree of plasticity in the nervous system in the integration of these essentially similar organs for very different habits of life.

TRANSLOCATIONS IN THE MOUSE AND THEIR EFFECT ON DEVELOPMENT

DR. GEORGE D. SNELL, ELSIE BODEMANN, AND WILLARD HOLLANDER
Department of Zoology, University of Texas

An experiment which has been in progress during the past two years has shown that when male mice are X-rayed with doses in the neighborhood of 600 Roentgen-units and mated to normal females, approximately one third of their progeny show induced heritable variations. By far the commonest of these variations consists of a tendency to produce small litters, usually comprising four young or less, instead of the usual seven or eight. For convenience this tendency towards small litter size has been termed semi-sterility, and evidence will be presented that it is due to the

presence of translocations induced by the x-ray treatment . Of 111 F_1 individuals from x-rayed fathers, 33 were probably semi-sterile, as against none in the control. Of these 33, 10 were selected for intensive study, first, of the inheritance of of the trait, second, of the embryological implications of the small litter size, and it is the results of this study that are reported below.

Up to the present time the genetic investigation has been confined to determining whether semi-sterile individuals transmit the trait to their progeny, and if so, to what proportion of their progeny. In the case of all ten of the semi-sterile stocks, it was found that the tendency to produce small litters was passed by the original semi-sterile individuals to a part of their descendants. Semi-sterile Male $F_1$146 gave the largest number of offspring and therefore is selected for further discussion. In accordance with the practice followed throughout the experiment, Male $F_1$146 was outcrossed to females of an untreated inbred stock. This rules out the possibility that his small litters were due to the segregation of recessive lethal genes. By these females he had 26 young in 7 litters, an average of 3.7 young per litter. This litter size differs significantly from the litter size of about 8 which is characteristic of these females when mated to normal males. Eighteen of these F_2 young were raised and again outcrossed to a normal stock to give the F_3. Three of these F_3's by a semi-sterile F_2 male were also raised and similarly outcrossed to make up a total of 21 individuals from the mating semi-sterile X normal. A number of litters, in most cases five or more, were raised from each of these 21 individuals to determine whether or not they had inherited, in whole or in part, the semi-sterility of their fathers. It was found that they fell into two groups, first, a group of 12 whose litters averaged 5.8 or less young, second, a group of 9 whose litters averaged 7.8 or more young. Twelve, therefore, were semi-sterile and nine normal. While these figures are not sufficiently extensive to establish the exact ratio of semi-steriles to normals occurring among the progeny of semi-sterile individuals, they are a satisfactory approximation to the 1:1 ratio which is expected on the assumption that semi-sterility is due to the presence of a translocation. Many additional data have been obtained which are in accord with the view that a semi-sterile individual transmits the trait to half its progeny, though no other one individual has been tested as thoroughly as Male $F_1$146.

Small litter size, proven by the above data to be a transmissible trait, has been shown in an embryological investigation to be due to the intra-uterine death of a portion of the embryos. In the case of most semi-sterile stocks the majority of nonviable embryos degenerate at about the time of implantation, leaving, however, swellings or solid moles which persist for six or more days and mark their location in the uterus. The remainder of the nonviable embryos develop further, usually showing at 12 to 14 days gross abnormalities in which the central nervous system is most conspicuously affected. The abnormalities can generally be classified as one of two types. The first of these is a type in which the neural tube, in whole or in part, fails to close over. Most commonly only the anterior end remains open, producing a monster in which that part of the brain normally forming the inner surface remains exposed to the outside. Less commonly the whole neural tube fails to close, resulting in complete spina bifida. In the second type the neural tube closes throughout its length, but becomes distended so that it has to fold or kink to adapt itself to the available space. Certain individuals of the first type occasionally come to term, but the majority die before term and are resorbed. Both of these types are produced by most of the semi-sterile stocks, and vary within wide limits according to the stock from which they come. Within any one stock, however, they show a certain amount of uniformity.

Of 971 embryos from 10 semi-sterile stocks, ranging in age from 10 to 14 days, 507, or 59%, were degenerate or abnormal, 67 being monsters. One semi-sterile stock, No. 109 produced 29 of these monsters out of a total of only 188 young, but all except two of the remaining nine stocks produced at least one monster. In a control group of 213 embryos, 30, or 13% were degenerate. Only 3 were monsters, these 3 all being of the distended central nervous system type.

In some of the semi-sterile stocks there is reason to believe that two or more translocations are present. This is indicated by the high percentage of degenerate embryos, and by the fact that a greater or less degree of semi-sterility is transmitted to considerably more than half the progeny. In the case of the others, however, less than one half of the zygotes are nonviable, about 38% in the case of stock 146, for example. This is in accord with the results obtained by Dobzhansky and Sturtevant. and by Glass, for reciprocal translocations involving the second and third chromosomes of Drosophila. These investigators have found that somewhat less than half of the gametes produced by an individual heterozygous for a translocation are of the types that give nonviable zygotes.

It is noteworthy that many of the abnormal human embryos described by Mall and other investigators are strikingly similar to the abnormal embryos produced by semi-sterile mice, and it appears probable that some, at least, of these types

of abnormalities in man are due to translocations and other chromosome aberrations.

To obtain further evidence as to whether or not semi-sterility in mice is due to the presence of translocations, cytological studies and linkage tests are at present in progress with the semi-sterile stocks.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 25.)

COMMENTS ON THE SEMINAR REPORT BY GEORGE D. SNELL, ELSIE BODEMANN, AND WILLARD HOLLANDER

P. W. Whiting
Professor of Zoology, University of Pittsburgh

Genetic evidence for chromosome translocations was shown several years ago by Bridges in Drosophila and the brilliant X-radiation work reported by Müller in 1927 has made possible more rapid analysis of chromosome constitution. Many investigators have followed Müller's lead so that we now have abundant evidence for translocations and other chromosomal irregularities not only in Drosophila but in several insects and in numerous plants.

To those who work with Drosophila, mammals seem painfully slow. For one who would attempt to demonstrate induced hereditary changes in a mammal, the mouse, however, is a happy choice. Small size requiring little space, rapid generations and large litters as well as the presence of several clear-cut genetic traits are assets which the thoughtful investigator may well prize.

Treatment of the males might be expected to give dominant lethal genetic effects as was demonstrated by Müller for Drosophila in 1927. Dr. Snell and his co-workers have actually shown these by decreased litter size, correlated with the presence of embryonic and fetal abnormalities. Demonstration of the hereditary tendency toward certain non-viable types in different lines is of interest not only to genetics but to medical science as well.

Dr. Snell appears to have done a very creditable piece of work with careful planning of experiments and accurate interpretation of results. Although we can hardly expect a demonstration of translocation as convincing as may be shown in Drosophila with its complete chromosome map, evidence that translocations lie at the basis of certain embryonic defects closely parallel with human conditions is a distinct contribution. We shall be interested to hear more in detail of this work.

CHROMATIN EXTRUSION IN CERTAIN CILIATE COMMENSALS OF MOLLUSCS

Dr. G. W. Kidder
Tutor in Biology, College of the City of New York

Certain ciliate commensals of lamellobranch molluscs seem to offer excellent cytological material for the study of macronuclear chromatin. I am going to describe briefly one phase of the macronuclear chromatin, that of the anlagen following conjugation.

First a review of the condition found in *Conchophthirius mytili* from the common mussel *Mytilus edulis*. This ciliate possesses in the vegetative state one large macronucleus and from one to four micronuclei.

After conjugation the amphinucleus divides rapidly four times resulting in sixteen apparently equal products. Of these sixteen, twelve to fifteen become differentiated, by swelling, into the macronuclear anlagen, while the remaining four to one become the functional micronuclei. As the old macronucleus is degenerating a peculiar change takes place in each anlage. Dense chromatin spheres are built up in the center and as the exconjugant prepares for its first cell division these spheres of chromatin migrate to the periphery and are cast out into the cytoplasm. Here they degenerate and are absorbed. The anlagen, in the meantime, have been segregated into two groups and pass to each of the daughter cells. In order to arrive at the vegetative state the ciliate must, of course, undergo a number of segregating cell divisions. At each division more chromatin is extruded into the cytoplasm, the amount decreasing at each successive division.

Ancistruma isseli from the solitary mussel *Modiola* behaves in a very similar manner. This ciliate always has one macronucleus and one micronucleus in the vegetative state. Only three amphinuclear divisions occur before differentiation takes place, resulting in eight apparently equal products. Seven of these swell and become macronuclear anlagen while one becomes the functional micronucleus, dividing at each cell division by typical mitosis. In this ciliate relative-

(*Continued on Page* 146)

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

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THE BIOLOGICAL LABORATORY AND THE COLLECTING NET

We hope that our subscribers read the announcement of Dr. Harris, director of the Biological Laboratory at Cold Spring Harbor, introducing his section of The Collecting Net. The acceptance of our proposal for publishing the lectures and seminars in The Collecting Net is a compliment which has made us happy. It is a partial realization of our plan that The Collecting Net might some day become the common organ of the many marine stations for biolo-by in the United States. A bigger step toward complete realization could not have been taken.

In his announcement Dr. Harris remarks that independent biological laboratories should exist and must function for all biologists, and that he welcomes the "valuable opportunity" of "any practicable means of making the Biological Laboratory, and the results of its work and conferences, more generally useful and available."

It is an especial privilege to be able to serve as a medium for the dissemination of the results of the important work of the Biological Laboratory. Dr. Harris adeptly emphasizes the importance of fostering a closer relationship between biology and the basic sciences: mathematics, physics and chemistry." As science advances it becomes more exact. Biophysics is a new science; the phrase "quantitative biology" would have been out of place at the beginning of the present century.

The Collecting Net began publication in 1926. For five years we were not called upon to use "π" or "$\surd$"; seven years passed before our authors required exponents and calculus characters. Is not this indicative of the trend of modern biology?

THE COLLECTING NET

In our last issue we made some general comments on the editorial policies of The Collecting Net, emphasizing the fact that we wanted to retain our "informality" and that we did not aspire to become an "accredited scientific magazine." These remarks were cut short by limitations of space.

It may be long before it becomes the custom for biologists to write informal articles about biological problems; discuss freely the work of their colleagues and introduce some of their own philosophy into the articles that they contribute. General essays on the bearing of modern biology upon the other sciences, upon philosophy and religion, indeed, upon any phase of human affairs, would be of great interest and perhaps of importance.

The cynical will remark that we will publish a lot of nonsense. Probably we will! But after all one worthwhile contribution would make up for a good deal of "nonsense."

DISTRIBUTION AND ECOLOGY OF THE MARINE ALGAE ON LAKE FISH!

Our increase in size has temporarily swamped our small printing plant in New Bedford, but we hope to return to our normal schedule soon. On more than one occasion we have discovered too late that galley proof has not been submitted to the author. We wish to tender apologies to authors to whom proof has not been submitted. Especially do we apologize to Dr. Bell. When his seminar report came to us it lacked its title which we added in New Bedford (just as the number was going to press) from the calendar in the previous number, the relevant portion of which we reproduce here:

> **Seminar: Dr. G. W. Prescott: "Some Effects of blue-green Algae on Lake Fish."**
> **Dr. Hugh P. Bell: "Distribution and Ecology of the Marine Algae of the Maritime Provinces of Canada."**

A glance at the lines will explain, though not excuse, the mistake.

Almost simultaneously, so it seemed to us, with the delivery of the issue in question to Woods Hole, there suddenly appeared two posters—one at the laboratory and one at the mess hall. We confiscated them for publication purposes and one of them appears on the opposite page.

The smoker held in the Clubhouse after Dr. Lucké's lecture on "The Zoological Distribution of Tumors" was attended by more than two hundred people who exchanged ideas and opinions among themselves over punch and cigarettes. This gathering afforded an excellent opportunity for many of the audience to meet and speak with the lecturer. The M. B. L. Club will hold a similar smoker after each of the Friday evening lectures. All members of the club (and those planning to join) are urged to come to these informal gatherings.

ITEMS OF INTEREST

Introducing

DR. ZENON M. BACQ, advance fellow of the Commission for Relief in Belgium Educational Foundation who is spending July and August in Woods Hole.

Dr. Bacq recently received the degree of "professeur agrégé" in physiology from the University of Liège for his studies on the humoral transmission of nerve impulses. His treatise on the subject appeared in the *Archives Internationales de Physiologie* for April, 1933.

He is investigating his problem from the viewpoint of comparative physiology. At the moment he is working on the humoral transmission of nerve impulses in the heart of the squid. He is interested in the subject of the innervation of the genital organs and is studying the reactions of the autonomic nervous system to drugs. When he leaves Woods Hole during the latter part of August he will go to Harvard University where he will consult with Dr. Walter B. Cannon, professor of physiology at the Harvard Medical School. He will return to Belgium late in October.

A graduate of the University of Brussels in 1927, Dr. Bacq visited this country in 1929, again under the auspices of the Belgium Educational Foundation, and worked with Dr. Cannon at Harvard during that year. He has recently been assisting Dr. Henri Fredericq, professor of physiology at the University of Liège, who worked at Woods Hole a few summers ago.—*R. G.*

THE MIGRATION OF TUNNY FISH

We recently received the following communication from the Commissioner of Fisheries of the United States Department of Commerce:

"The State Department has forwarded to the Department of Commerce a communication from the Portuguese Legation, advising of the continued studies by the research ship *Albacora* with respect to the migration of the tunny fish in Atlantic waters, and requesting that proper publicity be given to scientific and commercial institutions to the end that records of tagged fish taken in the North Atlantic might be obtained.

The Bureau of Fisheries will appreciate the publication of the enclosed note in the columns of your journal."

The Notes

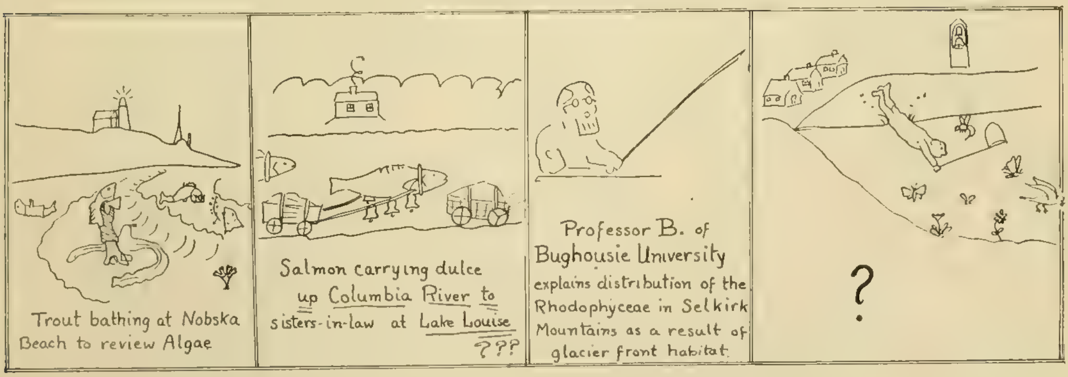
The Portuguese research ship "Albacora," employed in oceanographic investigations, has resumed studies on the migrations of tunny fish in the North Atlantic by marking 60 fish with metal disks bearing the legend "R. P. AQUARIO—LISBOA—PORTUGAL."

The cooperation of the oceanographic institutions and laboratories, and of the fishermen and fishing corporations has been requested by the Portuguese Legation in Washington to the end that the disks when found may be returned to the AQUARIO VASCO DA GAMA, LISBOA, PORTUGAL, with appropriate information regarding the day, hour and locality where the fish was caught. A reward is offered for such records of recapture.

DISTRIBUTION AND ECOLOGY OF THE MARINE ALGAE ON LAKE FISH

Dr. H. P. Bell

Professor of Botany, Dalhousie University



CHROMATIN EXTRUSION IN CERTAIN CILIATE COMMENSALS OF MOLLUSCS

(*Continued from Page* 143)

ly huge amounts of chromatin are extruded at the first cell division of the exconjugant and smaller amounts at each of the subsequent divisions until the vegetative condition is reached.

This summer I have been investigating the condition found in *Conchophthirius anodontae* from fresh water mussels. Although these observations are far from complete there seems to be a rather close agreement between the reorganizing forms and those I have just described. The vegetative individual has a single macronucleus and a partially imbedded micronucleus. After three amphinuclear divisions seven macronuclear anlagen and one micronucleus differentiate, as in *Ancistruma isseli.*. Spheres of chromatin form within each anlage, fuse and are extruded into the cytoplasm during the first exconjugant division. More extrusion chromatin is formed and thrown out in the next two divisions, as in the preceding species. The form of this later extrusion chromatin is slightly different but the result is the same.

As to the meaning of this phenomenon we can only speculate. If we consider the macronucleus of ciliates to be trophic in function (in some way regulating the cell metabolism) and the micronucleus as germinal (functioning primarily during conjugation and endomixis) then this extruded chromatin may possibly be the germinal substance being cast out in the purification of a trophic cell element, the macronucleus. The micronucleus must be considered as retaining the germinal substance and some trophic substance, or at least the potentiality for forming trophic substance, as we know the new macronuclei are built up from micronuclear material following conjugation or endomixis.

It seems to me that we can compare this extrusion chromatin to the degeneration products of amphinuclear divisions as reported in many other ciliates. In those cases it may be that the germinal material is eliminated all at once. To bring this idea into line with those forms in which none of the amphinuclear products degenerate and there is apparently no extrusion chromatin, we may suppose that the differentiating mitosis of the amphinucleus is at least qualitatively heteropolar, only trophic material going to the pole that will form the new macronucleus.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 18.)

News Items from Cold Spring Harbor

Dr. Eric Ponder of the Department of Biology of Washington Square College, New York University, has just returned from England, and is now in residence at the Laboratory.

Dr. Robert Gaunt, Professor of Biology at the College of Charleston, recently returned to the Laboratory, following his marriage to Josephine Howland. Miss Howland, who is the daughter of Dr. Howland of Schenectady, received the M. S. degree at Brown University in June. Both Prof. and Mrs. Gaunt are former students of the Laboratory.

Dr. Hugo Fricke, of the Biological Laboratory, will sail for England and the continent on August 5th. Dr. Fricke will attend and present a paper before the Third Congress of Experimental Cytology at Cambridge, and will visit his parents in Copenhagen.

Dr. Robert Chambers was here from Woods Hole for a few days, during which he presented a paper on "Intracellular Oxidation-Reduction Potentials" in the symposium of July 24.

Among those who have been visitors to the Laboratory since the last issue of The Collecting Net are: Dr. Robert H. Halsey of Post Graduate Medical College, Dr. L. R. Blinks of Rockefeller Institute, Dr. Cobum of the College of Physicians and Surgeons, Mrs. Janet Daniels, and Miss Capps of P. and S.

Dr. Hans Müller of the Department of Physics of Massachusetts Institute of Technology will return shortly to Cambridge. Prof. Müller has been a member of the group engaged in the conference on electrical potential differences at interfaces and their bearing upon biological phenomena. He is to give a course at M. I. T. during the remainder of the summer.

Three all-day symposia, the last of the formal program of the conference this year, will be given this week; one each on Monday, Wednesday and Friday; for titles and speakers see the July 8th issue of The Collecting Net.

A dancing and card party was held in the recreation room of Blackford Hall, Wednesday evening of last week.

THE COLLECTING NET

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STUDIES ON THE CYTOLOGY OF AMPHIBIA

Dr. Arthur W. Pollister
Instructor in Zoology, Columbia University

A study has been made of the cytoplasmic components in a variety of Amphibian tissues, nearly all types being represented with the exception of the striated muscle fibre and the neurone. The Kull, Kolatchev, and Benda methods were chiefly employed. Every type of cell was found typically to contain three types of formed cytoplasmic structures, that are sharply distinguishable from one another in their morphology, namely: chondriosomes, usually in the shape of separate unbranched filaments; Golgi material, always in the form of thin lamellae; and, in nearly every case, a pair of centrioles, small granules closely adjacent to one another.

The tissue cells studied thus far fall into one or the other of two schemes in the arrangement of these three cytoplasmic components. The first group contains cells that are physiologically unpolarized in that all the cell surface is similarly (*Continued on Page* 156)

CONDITIONS OF LIFE IN THE DEPTHS OF THE OCEAN

Dr. August Krogh
Professor of Zoophysiology, University of Copenhagen

Having undertaken to speak on this subject I think it the safest plan to admit at once that I *know* next to nothing about it. What I hope to do is to show that there is a real and important problem and to create an interest in it not only passing and academic but practical since I am speaking at the place which has the best opportunities in the world for work of this kind and the best brains to utilize them.

I propose to speak only of the open ocean where the influence of the land is negligible, where the phytoplankton produces from the surface down to say 200 meters an excess of organic material which furnishes the basis of animal life through all depths. Below 200 meters while some assimilation may be going on, the dissimilation is in excess and below 400 meters there is dissimilation only, by animals and bacteria but no assimilation by plants.

M. B. L. Calendar

THURSDAY, AUG. 10, 8:00 P. M.

Seminar: Dr. A. B. Dawson: "The absorption of colloidal carbon by the mesonephric epithelium of Necturus."

Dr. V. Schechter: "Morphological and electrophoretic effects of the galvanic current on Griffithsia cells."

Mr. K. Dan: "The electric charge on the surface of sea-urchin eggs."

Dr. R. W. Gerard: "Electrical activity of the brain."

FRIDAY, AUG. 11, 8:00 P. M.

Lecture: Dr. Josef Spek: "Die Protoplasmadifferenzierung der Eizellen wahrend der ersten Entwicklung."

TABLE OF CONTENTS

How does organic material and energy become available for the animals living below 400 meters? One thing to consider at the very beginning is the question of intensity of animal life at the different levels. It appears that the intensity of life below the surface, that is, below 200 meters, decreases greatly. The following figures obtained by Hentschel for nannoplankton organisms indicate the possible extent of this:

Surface	10,000 per liter
50 meters	9,000 per liter
100 meters	2,700 per liter
400 meters	260 per liter
2000 meters	50 per liter
5000 meters	15 per liter

This shows very clearly the decrease in number of these minute organisms with increasing depth. For the larger organisms there are no corresponding figures. But we ought to have them. As I shall show later on they are necessary for the solution of the problem and it is undoubtedly now possible to determine the *quantity* of plankton at great depths. On and in the bottom itself a highly varied fauna exists. Let us consider for a moment the conditions under which these animals live, confining ourselves for the present to the Atlantic Ocean. This ocean covers an area of 90 million square kilometers with a mean depth of about 4000 meters. The depth of over one-fourth of the area exceeds 5000 meters. The pressure increases one atmosphere for 10 meters—at the mean depth the pressure is 400 atmospheres. There is at this depth absolute darkness—except for a very faint light given off by organisms. The temperature is practically uniform –0° –3° C. Oxygen is available in sufficient quantity 5-6 cc. per liter. Currents are flowing at a very slow rate estimated at 25 to 100 meters per hour.

Let us now try to picture the effect of these conditions upon the fauna. In former days great stress was put upon the high pressure—it was supposed that this affected the organisms in many ways. Later investigations show that pressure does not matter very much. The water and the organisms themselves are slightly compressed—at 4000 meters about 2%. One might suppose that the pressure would increase the viscosity but there is no evidence of this. There is oxygen enough and light is not essential for most animal organisms. At the prevailing temperatures the metabolism decreases to about 1/5 of the value at 10° C. This is of importance because it means that animals can subsist on much less food than would be necessary at higher temperatures. The density of population on the bottom is unknown. It is to be concluded from the dredgings and trawlings of the various expeditions that the density must be low. When a trawl going over 10,000 square meters of bottom catches 200 specimens the haul is considered exceptionally successful and in such a haul 50 or more species may be represented. I would strongly urge the adoption of quantitative methods. The Peterson grab for taking bottom samples comprising a known area has been successfully applied—depths down to 1100 meters and will probably work at any depth. The working of trawl and dredges at great depths is uncertain and they may take only a small fraction of the population actually present but when on similar bottom certain large areas give on average much fewer specimens per haul than others, it must be legitimate to conclude that the average density of population is actually lower in the first. It is therefore almost certainly significant that in the *Challenger* expedition the average trawling on the Globigerina ooze gave in the Atlantic 21 specimens, in the Pacific 56 and in the Southern ocean 97. This points to a correlation with the intensity of surface life and such a correlation has been definitely established by Hentschel for the bathypelagic nannoplankton forms. When at the surface nannoplankton organisms number over 100,000, the number at 2000 meters is found to be over 100; if the number at the surface goes down to 5000, the population at 2000 meters will be less than ten. This relationship points to certain conclusions with regard to the food supply at great depths which we have now to consider.

We have two possible alternatives. It is agreed that all the food comes ultimately from the surface, but the manner of its coming down, whether in the form of organisms and excreta from organisms, or whether the organisms become dissolved and are then utilized by some forms, at least, of those living at great depths of the sea, has not been certainly determined. The first of these possibilities, that animal life depends directly upon organisms sinking down from above, is supported by those facts that were just cited. In spite of these, however, this assumption presents very great difficulties. When phytoplankton organisms sink down through the water, they do become dissolved, so that when they are sought at great depths one finds only the empty shells—only in a very few cases is there a little protoplasm left in the shells. At intermediate depths of 400-1000 meters, there is a comparatively large number of animals who feed directly or indirectly through smaller forms upon the phytoplankton, so that it seems impossible that there would be enough to support animal life at the bottom of the ocean.

There is a possibility that we have animals from 200-400 meters living directly on phytoplankton sinking down, other animals feed upon these as they die and sink and so on down through the lower levels. This would involve a definite and

very rapid falling off of the quantity of living material with depth, since by far the larger fraction of the energy and material available at each depth must be used up in the metabolic processes.

Usually only the plankton organisms have been considered as a source of food for animals at great depths, but it might very well be that the bodies of large animals living at the surface could constitute a signicant source of supply. Large fishes or whales, will at least sink down to the bottom fairly rapidly, while the plankton organisms sink extremely slowly—it would be a year or so probably before the latter reach the bottom if ever they get so far, but a large fish would reach a depth of 4000 meters in a couple of days. Recent admittedly very rough, calculations by Hjort give the number of whales in the South Sea in an area of 8 million square kilometers as 300,000. Assuming a population in a state of equilibrium this would mean that about 50,000 whales would die and sink down to the bottom in this area every year. If that were so, it would mean one whale for each 160 square kilometers, corresponding to 50 square miles, each year. This does not sound like much but it would amount really to a great deal, about half a gram available organic material for each square meter. So it is possible that the bodies of large animals may constitute an amount of food for the animals at the bottom that is not at all insignificant.

Many animals at great depths are adapted to have food only at rare intervals. Several deep sea fishes can swallow a prey of their own size or larger and if such a fish had food once a year, that might be quite sufficient. Even at 15° C. many fish and other animals can live without food for six months, and at lower temperatures this period would be extended. There is not complete data at present to indicate whether animals sinking down to the bottom constitute a sufficient or insufficient food resource. Some years ago it was maintained by Pütter that this source of food and excreta would be absolutely insufficient and must be supplemented by dissolved organic material.

There is no reason to believe now that for most animals the dissolved organic material is essential. Still, there is a possibility that for certain organisms, at least, dissolved organic material might constitute a very important item.

Information as to the organic material present in solution in the ocean is admittedly incomplete and the older determinations are unreliable. I have made a series of determinations on samples taken in the Mid-Atlantic. These samples showed very little variation and an average amount of 244 mg. N per cubic meter, and of 2350 mg. C. per cubic meter. These figures are probably not quite right, since the water samples had been standing quite a while before they were analysed, thus causing a possible decrease. We are making new determinations now that should be more accurate. But whether the figures are a little high or low does not matter much, it is evident that the amount of organic material in solution is enormous. I have tried to figure out the amount present in organisms at any one time. It seems that this is only about 1/1000 of the total supply. This leaves a very large surplus available but it is a question whether it can be utilized. There is some reason to believe that a very large part of it cannot be utilized even by bacteria. If it were to constitute a source of supply for very many bacteria, we should find them in far greater numbers than we now do. As a matter of fact, these substances, whatever they may be, are not very good food. It can be shown that all large animals, like all the fishes, crustacea, etc., are unable to take up organic material from solution in any significant amount. Bacteria, protozoa, and perhaps a few other forms have such a large surface compared to their volume that they would be more likely to manage to use it directly.

Alexander Agassiz maintained fifty years ago that there must be at the bottom of the ocean a very large number of protozoa. And they may be there, but no one so far has seen them. But if they are there, they might live on dissolved material and themselves then constitute a source of food for the larger animals. It seems possible also that sponges might be able to utilize dissolved material to a significant extent. Sponges seem to be relatively abundant in certain places at great depths and it is not easy to see what else they can get.

It is however quite certain that food must be available in the bottom and just above the bottom in the form of minute particles, but where these particles come from is very difficult to say. Numbers of animals at all depths live in mud. We have observed during the recent cruise of the *Atlantis* that even at the greatest depth the surface of the bottom is largely made up of fecal pellets, showing that animals eat mud and must be supposed to subsist on it. Even this conclusion is a little dangerous. I have made experiments on mud eating animals living at shallow depths where the mud contains a large amount of organic material. Samples of such mud were analyzed and the amount of organic material in it determined. Then different animals were kept in a known amount of the mud for a fortnight or a month. Considering the oxygen used by the animals, they should have used one-fourth to one-half of the organic material in the mud. As it turned out, there was exactly the same amount present in the mud as before. There is still reason to believe, however, that these animals do not in natural con-

ditions eat the mud indiscriminately, but rather the material flowing along its surface.

I think we must face the possibility that there may be at the bottom at great depths, micro-organisms which live on dissolved substances, and which constitute an important source of ultimate food for higher animals. Personally I believe that other possibilities are perhaps more likely.

It will be evident to all here that I have said very little—given very little definite information about what is going on at great depths. What I have tried to do was to present the problems that are still to be solved, which I hope may stimulate investigation in this direction.

In conclusion I should like to give a quotation from a great countryman of mine, Niels Stensen or latinized Nicolaus Stenonius born just 300 years ago. In his inaugural address in the Copenhagen Anatomical Theatre he said:

"Beautiful are the things we see,
More beautiful those we understand,
By far the most beautiful are those of which
We are still ignorant."

STUDIES ON THE CYTOLOGY OF AMPHIBIA

(*Continued from Page* 153)

exposed to the surrounding tissue fluid. An example of this is the leucocyte. Under these conditions the organization of the cytoplasm is very definitely focussed in the centrioles. These may be enclosed in a specialized area of the cytoplasm, the centrosphere, and with strong fixing fluids one can demonstrate in cells with a considerable volume of cytoplasm, an extensive aster, the rays of which are radial to the region of the centrioles. The Golgi material is in the form of an irregular membrane surrounding the central apparatus. In cells with a prominent aster the chondriosomes in its vicinity are oriented with their long axes parallel to the astral rays, i. e., radial to the centrioles. In other regions of the leucocyte and in cells without an aster the chondrioconts are oriented quite at random. I have found that the following cells belong to the physiologically unpolarized type, in which the Golgi apparatus is closely aggregated about the centrioles: leucocytes, erythrocytes, mesenchyme cells, fibroblasts, gonial cells, peritoneal epithelial cells, endothelial cells, and smooth muscle fibres.

The second cell type comprises the polarized epithelial cells, which have one surface in contact with a cavity or lumen, the opposite surface wholly or partly in contact with the tissue fluid, while the other sides are contiguous to adjacent epithelial cells. These external influences determine the orientation of the cytoplasmic components, replacing the possibly more fundamental or intrinsic tendency toward an organization about the centrioles. The most striking morphological difference between the polarized and unpolarized cell is the fact that in the former the Golgi material has no fixed close topographical relationship to the centrioles. The latter are usually located near the center of the distal end of the cell. The axis passing through the two is often nearly perpendicular to the cell surface and, in some types, a flagellum is attached to the distal centriole. The Golgi material in most cases forms a thin irregular belt that often surrounds the distal end of the nucleus. In some instances it is in the form of separate small lamellae in the same position as the other type. In glandular cells the Golgi material is localized at the site of synthesis of the secretory product. In all the polarized cells the chondrioconts are oriented so that their long axes are approximately parallel to the direction of the flow of material through the cell, i. e., between the base and the lumen. It is suggested that cytoplasmic currents are responsible both for this arrangement and for the radial orientation of chondriosomes in the unpolarized cells.

(This article is based upon a seminar report which will be presented at the Marine Biological Laboratory on August 1.)

THE BIOLOGICAL LABORATORY
COLD SPRING HARBOR

STABILITY OF COLLOIDS AND THE THEORY OF RAPID COAGULATION

Hans Müller

The small degree of stability of certain colloidal solutions is well known to every experimenter. Small amounts of electrolyte may produce coagulation. Small changes in temperature or changes in the dielectric constant of the solvent may produce the same effect. Some colloids, like vanadiumpentoxyde, show "aging" effects; the properties of these colloids change with time. These facts raise serious doubts as to whether a colloid may be considered as in the state of thermodynamical equilibrium. The colloidal state is, perhaps, only a state of transition, a pseudoequilibrium with a relatively long lifetime. On the other hand, there exist colloids which have a degree of stability as high as any chemical compound. We are, therefore, at least in some cases, justified in considering the colloidal state as a thermodynamical equilibrium. The laws of thermodynamics should, therefore, be able to explain why a colloid can exist, and furnish the conditions under which coagulation occurs.

Unfortunately, a satisfactory theory of the stability of colloidal solutions encounters great difficulties. Let us consider a simple colloid, such as, for instance, a gold colloid. The micelles are small crystals of gold. The exact value of the surface tension γ between gold and water is not known, but it must be of the order of magnitude of 10 to 1000 erg/cm^2. Let us assume a cubical form of the particles, the edges of the cube having the length r. If two such particles grow together along the sides of the cubes, two surfaces each of the area r^2, vanish, and the surface energy $2r^2\gamma$ becomes free. According to the second law of thermodynamics a system is in equilibrium if its free energy is a minimum. The surface tension acts, therefore, in such a way as to produce coagulation. The temperature motion, however, acts to prevent this process. Whether coagulation takes place or not depends on whether the energy of the temperature motion is larger or smaller than the decrease of surface energy. Hence a colloid should be stable only if

$$2\gamma r^2 \leqq {}^3/_2\, k\, T$$

Introducing $k = 1.37\ 10^{-16}$ erg, $T = 300$ and an average value of $\gamma = 100$ gives

$$r \leqq 1.7\ 10^{-8}\ \text{cm.}$$

Even if we assume the surface energy to be 10 times smaller, we find that a gold colloid should only be stable if the particles consist of but a few gold atoms.

To be conclusive, this elementary consideration requires, of course, a more rigorous derivation. One has to find the conditions for which the free energy of the colloid is a minimum. This was done in a paper by March [1], and he comes to the same conclusion as that found above. From this theoretical point of view a stable colloid with a particle size larger than 10^{-7} cm. is thermodynamically impossible. Experimentally, however, we find colloids with particles of 10^{-5} cm. radius, which are apparently stable.

There are two important points which have to be considered for an explanation of this discrepancy between theory and fact. The first point concerns the value of the free surface energy. The surface energy of a solid-liquid interphase is not determined by the surface energy of the liquid and the solid phase. The molecules on the surface of the solid exert forces which act on the molecules of the liquid. This leads to solvation and a smaller value of the surface tension. It is conceivable that in some colloids the hydration reduces the surface tension to such a low value that very large particles are stable. But these cases are exceptions. In general the surface tension will have values of the magnitude assumed above.

More important is the relation between stability and the electric potential of the micelles. The experiments point to the fact that coagulation occurs if the electrokinetic potential is diminished below a critical value. It is, therefore, evident that the factor determining the stability of a colloid is primarily determined by the charge of the particle and by the constitution of the electric double layer. The reason for this correlation seems to be simple: if two particles approach each other they are repelled by the Coulomb forces acting between the charges of the double layer. These forces constitute a repulsion, since the charge of the outer layers have the same sign for the two particles. Unfortunately the calculation shows that these repulsive forces are not strong enough to balance the attraction due to the surface tension. If the energy of the electric double layer is taken into account in March's calculation, the

stability of somewhat larger particles can be explained, but the micelles should still be of amicroscopic size in order to be theoretically stable. A similar calculation of Gyemant [2] also leads to the stability of particles with a radius of less than 10^{-6} cm. only.

Since stable colloids with ultramicroscopically and even microscopically small particles do exist, we must conclude that the present theories have neglected a source of negative free energy. This neglected energy must be connected with the electric field around the micelles.

The origin of this energy may possibly be explained in the same way in which Zwicky [3] explains the anomalous specific heat of strong electrolytes. While Debye's and Hückel's theory of strong electrolytes gives an adequate explanation of the mobility and activity of the ions, it fails to explain the caloric properties of electrolytes. It has been observed that the specific heat of strong electrolytes is considerably smaller than the specific heat of pure water. The interionic forces give rise to a small decrease of the specific heat, but they cannot account for the observed, large variation. In some cases the variation is so large that the heat necessary to change the temperature of a solution containing 1 liter of water and 100 gr. of salt is even less than one calorie.

According to Zwicky, this anomaly is due to the strong electric field existing in the neighborhood of the ions. This field polarises the water and gives rise to electrostrictive forces. The electrostriction produces a hydrostatic pressure of many thousand atmospheres. According to measurements of Bridgeman, the specific heat of water decreases with pressure. Around every ion there is, therefore, a shell of water, whose specific heat is reduced by the electrostrictive pressure. Zwicky was able to show that this effect can account for the observed decrease of specific heat of the solution. The electric field existing near the surface of colloidal particles is of the same order of magnitude as the field around ions. The water in the double layer of a micelle is, therefore, under a large pressure produced by electrostriction. Since pressure reduces the specific heat of water, the water in the double layer has a smaller free energy than the water in the solution. The calculation shows that this decrease of free energy is sufficient to explain the stability of large colloidal particles.

This consideration verifies, to a certain extent, the conclusion of March. March states that one must assume the existence of a protecting skin around each micelle. This protective skin is here explained as a layer of water, under high pressure. If the thickness of the double layer is diminished, by addition of electrolyte, the protective skin gradually disappears, and coagulation begins.

It seems probable that this consideration may contribute to the understanding of the action of protective colloids, and the coagulation by nonelectrolytes. If the electrolyte concentration of a colloid is gradually increased, until the ζ potential decreases to a critical value, slow coagulation begins. In this state only particles with a large kinetic energy are able to overcome the stabilizing forces. If the ζ potential is made sufficiently small, all impacts between particles result in joining them. We have, then, the maximum rate of coagulation, or rapid coagulation.

In his last paper before his death in 1917 v. Smoluchowski [4] gave the mathematical theory of rapid coagulation. The results of this theory were repeatedly verified by Zsigmondi [5], Westgren and Reitstötter [6], Kruyt and van Arkel [7] and Tuorila [8]. Theory and experiment agree extremely well. Both lead to the conclusion that the rate of coagulation is independent of the size of the particles. This result seems, however, to be in contradiction to measurements of Wiegner [9] and Galecki [10], who found, even before the theory was developed, that small particles join, preferably, large particles. They observed, namely, that the amicroscopic particles in milk do not group together to form large particles, but that they attach themselves to the larger particles in the solution.

Following a suggestion of Dr. Wiegner, I studied this apparent contradiction to Smoluchowski's theory, and I succeeded subsequently [11], not only in clearing up this question, but in giving a more generalized theory. It is now possible to calculate the rate of rapid coagulation of any colloid with arbitrary numbers of arbitrarily large, and arbitrarily shaped, particles. The conclusions of this generalized theory have been experimentally verified by G. Wiegner [12], P. Tuorila [13] and C. E. Marshall [14].

The theory of rapid coagulation is based on the following assumptions: (1) Every impact of any two particles results in joining them together. (2) The impacts are governed by the laws of Brownian motion. There are no other forces, besides the temperature energy, which produce, or prevent, impacts.

The calculation proceeds in two steps. The first step consists in calculating the number of particles of a certain type which collide during an infinitesimal time dt with an arbitrarily chosen particle. This number dn can be given by an equation

$$dn = 2npdt \qquad (1)$$

where n is the number per $cm.^3$ of particles of the considered type in the colloid at the time t, and p is a measure of the probability of occur-

rence of an impact. The main problem consists in calculating this probability p.

The second step consists in counting the number of disappearing and newly formed conglomerates of particles. This leads to a system of infinitely many differential equations, with an infinite number of variables, but the solution of the equations is always simple, and leads to the result that the total number N of all particles decreases with time according to the formula

$$\frac{1}{N P} \cdot \frac{d N}{d t} = - N \qquad (2)$$

Here P is an average value of the probabilities p of all possible impacts between all the different types of particles existing in the initial colloid, and all the possible conglomerates of particles formed during the coagulation. Since the distribution of sizes and shapes of the micelles changes during the course of the coagulation, the value of P changes with time. If P is considered as a constant, equation (2) can be integrated and gives

$$N(t) = \frac{N_0}{1 + \frac{t}{\tau}} \qquad (3)$$

where

$$\tau = \frac{1}{N_0 P} \qquad (4)$$

and where N_0 is the total number of particles before the beginning of the coagulation.

τ is called the half-time of coagulation. τ seconds after the beginning of coagulation, the total number of all particles has decreased to half its original value. After 2τ, 3τ, 4τ . . . seconds the total number has fallen to 1/3, 1/4, 1/5 . . . of its original value. The shorter the time τ, the faster the rate of coagulation. According to equation (4) the rate of coagulation is the faster the larger the concentration of the colloid, and the larger the average probability P.

It can be shown that the value of P changes in many cases very slightly. The results (3) and (4) are applicable for nearly all colloids.

In order to calculate the probabilities p . . . . one has to find the number of particles of a certain type i which collide during the time dt with a particle of another type k. For the sake of simplicity, we consider first particles of spherical shape with radii r_i and r_k, respectively. A particle of type i collides with the particle of type k if their centers are at a distance $(r_i + r_k)$. Since we assume that all impacts are due to Brownian movement, one has to solve a problem of diffusion, namely, one has to find a solution of the differential equation of diffusion

$$\frac{d\, n_i}{d\, t} = D \, \Delta \, n_i \qquad (5)$$

Satisfying the boundary condition $n_i = 0$ for $r = r_i + r_k$, and $n_i = n_i^0$ for $t = 0$. Here D is the sum of the diffusion constants of the two types of particles, and has, according to Einstein and Smoluchowski, the value

$$D = \frac{k T}{6 \pi \eta} \left(\frac{1}{r_i} + \frac{1}{r_k} \right) \qquad (6)$$

where k is Boltzmann's constant, T the absolute temperature and η the viscosity of the colloid.

It is easy to realize how the diffusion of the i particles will proceed. Shortly after the beginning of the coagulation all particles of type i in the neighborhood of the k-particle will have made an impact with it. This initial rush will slow down, and the number of impacts will assume a steady rate, determined by the rate with which the coagulating particles are replaced by diffusion of the i-particles further away from the k-particle. This result is verified by Smoluchowski's calculation. One finds that the initial rush is completed after such a small time, that it is of no importance for the course of the coagulation. Consequently, one has to find only the steady rate of diffusion. The time element can, therefore, be eliminated,

$$\frac{d\, n_i}{d\, t} = 0,$$

and instead of (5) we have a much simpler differential equation

$$\Delta \, n_i = 0 \qquad (7)$$

whose solution has to satisfy the boundary condition $n_i = 0$ for $r = r_i + r_k$, and $n_i = n_i^0$ for $r = \infty$.

Equation (7) is Laplace's equation. The same equation holds for the electric potential distribution around a charged conductor. The solution of the diffusion problem can, therefore, be found in the analogous problem of electrostatics. This analogy shows that the number of colliding particles is proportional to the number of electric lines of force in the corresponding electrostatic problem, and the probability p is given by

$$p = 2 \pi D C \qquad (8)$$

where C is the electrostatic capacity of the surface, which the centers of the i-particles must reach in order to collide with the k-particle.

For spherical particles this surface is the sphere of radius $(r_i + r_k)$. Since the capacity of a sphere is equal to its radius, we have for spheres

$$p = \frac{(r_i + r_k)^2}{4\, r_i\, r_k}\, 4/3\, \frac{k\,T}{\eta} \qquad (9)$$

If $r_i = r_k$ this reduces to

$$p_o = 4/3\, \frac{k\,T}{\eta} \qquad (10)$$

If, therefore, a colloid contains spherical particles of uniform size, the probabality p_o, and hence also the average value P is independent of the size of the particles. This independence of size is due to the fact that a particle of large radius presents a large target for impacts, but has also a small velocity. These two influences of its size just cancel each other. Smoluchowski's conclusions are based on this result.

If however, $r_i > r_k$, then, according to (9) the probability, p is larger than p_o. This fact explains the observation of Wiegner and Galecki. A small particle has a much greater chance to collide with a large particle, than it has with one of its own size. The large particles form nuclei of coagulation for the small ones. The rate of coagulation of a sol with small micelles can be increased by adding a small number of large particles. I have given the complete theory of the influence of large particles on the coagulation of small particles. P. Tuorila has found that all conclusions of the theory are verified. Table I shows how the measured number of particles decreases according to my theory and not according to Smoluchowski's.

Table I

Coagulation of a mixture of two gold sols, one having $N_o = 3.6\ 10^8$ particles of radius $97\mu\mu$, the other $n_o = 29282$ particles of radius $2.91\ \mu\mu$, according to measurement of G. Wiegner and P. Tuorila.

Time t	$(N + n)\ 10^{-8}$ observed	$(N + n)\ 10^{-8}$ Calc. Müller	$(N + n)\ 10^{-8}$ Calc. Smoluchowski
0	29282		
120	4.5 ± 0.1	4.4	15.2
240	3.3 ± 0.2	2.5	7.6
480	2.1 ± 0.1	1.9	3.8

The influence of different radii is only pronounced if the radii differ by more than a ratio 1:10. Furthermore, it is necessary that the large particles are just as numerous as the small ones. In an ordinary colloid the particles are usually not of a uniform size, but the radii vary between two limits, most particles having a radius near the average value. Such a colloid shall be called "practically" monodisperse. It can be shown that for such a colloid, p and P are practically equal to p_o, and consequently they follow Smoluchowski's law of coagulation

$$N = \frac{N_o}{1 + \frac{t}{\tau}} \qquad \tau = \frac{3\,\eta}{N_o\, 4\, k\, T}$$

The possible deviations are smaller than the error of observation. This is the reason why most measurements verify Smoluchowski's curve, in spite of the fact that the assumptions of his theory are not justified for most of these investigated colloids.

If, however, the particle sizes vary greatly, as, for instance, in a mixture of colloidal solutions, large deviation must be expected. The coagulation proceeds faster than according to Smoluchowski's theory.

Using equation (8) it is possible to investigate the rate of coagulation of particles of arbitrary shape. It leads to the conclusion that particles of the shape of flakes coagulate in practically the same way as do spherical particles. The data on Kaolin verify this result. Rod-shaped particles, however, should have a much higher speed of coagulation than spherical particles. This conclusion was verified by Wiegner and Marshal [14], who observed a rate of coagulation of a $V_2\,O_5$ sol more than 30 times larger than Smoluchowski's theory predicts.

The theory of rapid coagulation has also been extended to colloids in the state of sedimentation. If large particles drop under the influence of gravity, through a coagulating colloid of small particles, they collide with the small micelles and carry them along. This phenomenon has been studied theoretically and experimentally by P. Tuorila [15]. I have given a somewhat different derivation, but both theories give the same results and agree equally well with the observation. It is found that this effect increases with the size and the number of the sedimenting particles. But, however large these particles may be, they do not affect the coagulation of particles smaller than a definite size. Gold particles are never cleaned out by sedimenting large particles, if they have a ra-

dius smaller than 300 $\mu\mu$. Quartz particles must have a radius of 500 $\mu\mu$.

The same effect can also be produced by stirring, or centrifuging, the colloid. These operations will increase the rate of coagulation if the colloid contains particles of different sizes; but the coagulation of particles smaller than a definite size will not be influenced by these mechanical means. In order to show this effect of "orthokinetic" coagulation the particles must be larger than

$$r \geq \sqrt[4]{\frac{1.2\,k\,T}{\pi\,a\,s}}$$

where s is the density of the particle material, and a the acceleration produced by the mechanical operation.

If the acceleration is very large, the coagulation may even still be produced if the electrolyte concentration is so small that the colloid would otherwise be stable. Whether this mechanical coagulation is simply an accelerated slow coagulation, produced by the above effect, or an entirely new phenomon, is at present difficult to decide.

Nearly all of the conclusions reached for rapid coagulation can be extended to the theory of slow coagulation. Slow coagulation differs from the rapid one insofar as only a certain percentage of impacts results in joining the particles. Consequently, the observed curves for slow coagulation are similar to those for rapid coagulation, but the coagulation time τ is increased by a factor depending on the concentration of the electrolyte. The dependence on electrolyte concentration was given in a theory by H. Freundlich [16].

The kinetics of the coagulation of colloids is, therefore, rather well understood. The problem, why and how a colloid is destroyed, is practically solved. The more important problem, however, why can a colloid exist, is still a matter of further investigations.

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Discussion

Dr. Cole: You mentioned that the coagulation of small particles is not affected by the sedimentation of large particles. Is this an experimental fact alone, or does it also follow from the theory?

Dr. Müller: It is a consequence of the theory and is verified by observations. The small particles make no impacts with the large ones, because the hydrodynamic currents around the falling particles carry the small particles away from the path of the large micelles.

Dr. Chen: In the coagulation by mechanical means, such as shaking, stirring and bubbling air through the solution, do you take into consideration the increased influence of the surface beside the increase of number of collisions among the particles? This kind of coagulation seems to depend upon the total surface of the colloidal solution.

Dr. Müller: The present theory neglects the coagulation taking place on the surface. In the theory for systems in the state of sedimentation by gravitation or centrifuging, this effect can be neglected. In other methods of mechanical coagulation, however, the surface effect can be much more important than the collision effect.

Dr. Blinks: Have any studies on coagulation of particles suspended in gases been made from this point of view?

Dr. Müller: Smoluchowski's theory has been used for the theory of the condensation of fog.

Dr. Cole: According to your theory the stability of a colloid depends largely on the radius of the particles. If, therefore, electrolyte is added to a sol one might expect that the larger particles should first become unstable, while the smaller ones might still be stable.

Dr. Müller: Investigations in Dr. Wiegner's laboratory show that this is the case. It was frequently observed that the large particle of a polydisperse sol underwent slow coagulation, while the small micelles were not flocculated.

Dr. Cole: Can the electrostriction effect influence the surface tension in the capillary electrometer?

Dr. Müller: This effect is only appreciable if the surface has a large curvature. It should, therefore, play no role in the capillary electrometer.

THE ELECTRIC POTENTIAL AND CHARGE OF DISSOLVED AND ADSORBED PROTEINS

Harold A. Abramson

The study of the electrophoresis of dissolved proteins and of protein surfaces is of interest not only from the point of view of their role in biological systems, but also because the theories of electrophoresis may be tested. I shall in this lecture attempt to review briefly some recent contributions to these aspects of the physical chemistry of the proteins.

Comparison of Dissolved and Adsorbed Protein

Early data dealing with the electric mobility of the proteins included not only measurements on the moving boundaries of dissolved proteins but also on very fine suspensions of protein particles or of inert particles having adsorbed protein surfaces. Indeed it was apparent that the surfaces of denatured proteins behaved very much like the dissolved molecules themselves although no quantitative comparison was made for dissolved protein and the protein surfaces except at the isoelectric point[1].

Loeb[2], for example, performed numerous experiments showing that there was approximate agreement between the isoelectric points of gelatin and of egg albumin when in solution and when adsorbed on collodion. This identity of isoelectric points is a necessary condition, but it is not sufficient to establish the fact that protein adsorbed on inert surfaces apparently ionizes exactly the same as the dissolved protein. In Figures 1 and 2 the open circles are mobility data of Tiselius[3] on dissolved egg albumin and serum albumin in M/50 acetate buffer. All the other points have been obtained by the writer[4] on microscopically visible quartz particles covered with protein and suspended in the same buffer. The quartz particles are about 1000 times the size of the dissolved protein molecules but, when a surface layer of adsorbed protein has formed, the particles covered with protein move with practically the same speed as the individual molecules themselves. These data indicate then that the implication in Loeb's experiments, that adsorbed protein had properties similar to dissolved protein, was justified.

From this point on we shall accept the experimental fact that in the case of egg albumin and serum albumin the electric mobility of the single protein ion and an inert surface covered with the

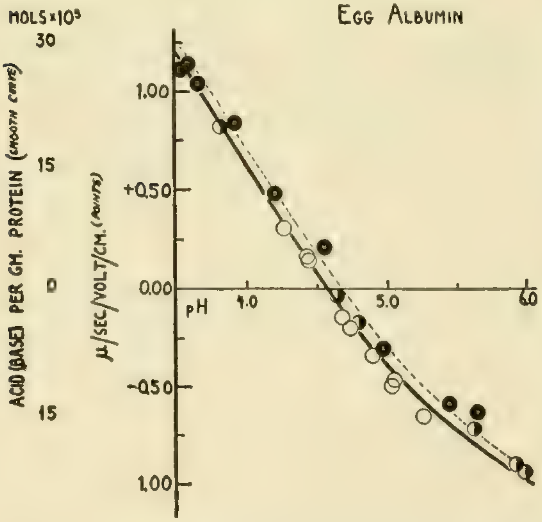

FIG. 1. The open circles are values of electric mobility of **dissolved** egg albumin obtained by Tiselius. The closed and half-closed circles are similar data for egg albumin studied under similar conditions but **adsorbed** on microscopically visible quartz particles. It is evident that the mobility and titration curves belong to the same family, so that over this range of pH, mobility is proportional to the acid (base) bound. The dotted line indicates the very slight shift in electrophoretic mobility between adsorbed and dissolved protein.

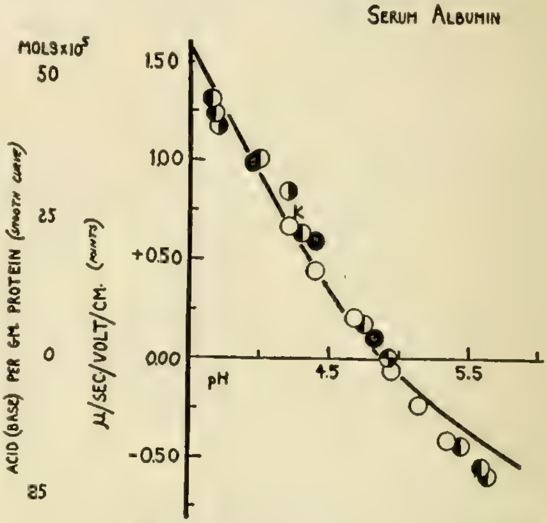

FIG. 2. The open circles are values of electric mobility of **dissolved** serum albumin (Tiselius). The other points are the mobilities of microscopically visible quartz particles covered with an **adsorbed** film of the same protein. There is no difference in mobility between the native dissolved protein molecules and the adsorbed protein. The heavy curve is the titration curve of a sample of serum albumin.

protein have approximately the same ζ-potential. In consequence we can extend our knowledge of the behavior of these proteins by the relatively simple microscopic technic of electrophoresis and thereby analyze more minutely the changes undergone by the protein ions incidental to adsorption.

Denaturation and Flocculation

The data on egg albumin and serum albumin can be used to interpret part of the process of denaturation in surface films. It is probable that a polymolecular film of protein is present at tne interface of quartz and liquid, first a monomolecular layer and then successive layers being added. The data probably describe the behavior of the outermost layer. If denaturation occurs at the interface, except for the extremely small shift of the isoelectric point of about 0.05 of a pH, at the limits of the experimental error, no other important change seems to have occurred in the total charge of the outermost molecules of egg albumin in contact with the liquid. Since measurements of mobility were made soon after the suspension of the quartz particles in the protein solutions, and since it is not impossible that in this type of denaturation the chemical process is a slow one, if a greater and sufficient length of time had been permitted to elapse, a more marked change might have been observed.

The identity of the values for the electrophoresis of dissolved native serum albumin and adsorbed albumin are of importance in connection with data of Pedersen[5]. Pedersen has found that serum albumin, after heat denaturation, has an isoelectric point between pH 5.1 and pH 5.3. The mobility-pH curve was nearly parallel to that of the native protein. If the denaturation occurs at a surface incidental to adsorption, as in the experiments reported here, this change in the isoelectric point and the mobilities at different values of pH does not occur. There is then, a very great difference between "*surface denaturation*" by inert particles and *heat denaturation* of the type used by Pedersen, in terms of the charge of the protein.

The Charge of Proteins by a Thermodynamic Method.

Although yielding no information concerning the mechanism of charge and primarily investigated for dissolved proteins rather than protein surfaces, measurements of the activity of the ions in a solution of a protein in an acid (base) can, under certain conditions, reveal the net charge of the protein. Given a very dilute protein solution in HC1 for example,

$$[H^+]_{Pr} + [H^+] = [Cl^-] + [Cl^-]_{Pr}$$

where the subscript Pr refers to the ions bound by the protein and the brackets represent concentrations. If n is the time average of the net charge,

$$n = [H^+]_{Pr} - [Cl^-]_{Pr}$$

and the charged protein molecule can be represented as Pr^{n+}. The activities of these ions when the solution is sufficiently dilute, say of egg albumin *near* its isoelectric point, can be taken equal to their concentrations so the measurements of a_{H^+} and a_{Cl} by the usual method before and after addition of protein gives the reduction in the number of ions of H^+ and Cl^- in the solution due to the presence of protein. St. Bugarsky and Lieberman, Manabe and Matula, Pauli and co-workers, Loeb, and Hitchcock early showed that, for proteins having isoelectric points close to pH 5.0, dissociation of protein chlorides (and other protein salts) was practically complete near the isoelectric point on the acid side[6]; that is

$$[Cl^-]_{Pr} \rightarrow O,$$

and that therefore,

$$n = [H^+]_{Pr} \ .$$

With this condition, the titration curve of the proteins portrays in the case of soluble proteins having known molecular weights the time average of the net charge per molecule. The problem attains a greater complexity at the ends of the titration curves where the activities and concentrations of ions are no longer equal. Evidently, the expression,

$$[Cl^-]_{Pr} = \frac{a_{H^+}}{\gamma_{H^+}} - \frac{a_{Cl^-}}{\gamma_{Cl^-}} + [H^+]_{Pr} = [Cl^-]\ \text{Total} - \frac{a_{Cl^-}}{\gamma_{Cl^-}}$$

measures the bound chlorine. By making use of this expression and assuming $a_{H^+} = a_{Cl^-}$, and that the presence of highly charged protein ions can be neglected, an assumption which is somewhat daring in solutions of proteins where the net charge is about 20, it can be demonstrated that proteins like some of the albumins and globulins are at least 85% dissociated in more acid solutions where the concentration of salt and of acid and of protein is considerable. Failey has recently considered the problem from the point of view of complete dissociation.

Failey measured the solubility of thallous chloride in solutions of nitric acid containing varied amounts of edestin. Without assuming any combination of P^{n+} and Cl^-, he found that the mean activity coefficient of the ions of the salt is de-

creased by the protein. Using these data and thereby correcting for the effect of the protein on the mean activity of the ions of HCl in the presence of protein it seems likely that gelatin, casein and edestin have a definite maximal combining capacity for H^+, with a vanishingly small combination of Cl^-. By these thermodynamic means it is possible to obtain values of the charge of protein molecules which may be used as references for testing the electrophoresis theory.

Mobility and Titration Curve of Proteins

By combining the Smoluchowski-Henry theory

$$v = \frac{1}{6\pi} \frac{\zeta_E D}{\eta} \qquad (1)$$

with the Debye approximation,

$$\zeta = \frac{\underset{\sim}{Q}\,{}^1/\kappa}{Dr\,(r + {}^1/\kappa)} \qquad (2)$$

where v = the electric mobility; ζ_E = electrokinetic potential; D = dielectric constant; η = coefficient of viscosity; $\underset{\sim}{Q}$ = net charge; r = radus, when

$$\frac{\zeta \mathbf{e}}{kT} < 1,$$

$\mathbf{e} = 4.77 \times 10^{-10}$ E. S. U. of charge, k = Boltzman's constant, and T the absolute temperature. Since $\kappa = 0.33\sqrt{C} \times 10^8$ at 20° in a solution of a uni-univalent salt of c moles per liter. In the experiments given in Figs. 1 and 2 the ionic strength was kept constant, varying aH^+, so that with this condition and combining equations (1) and (2),

$$\underset{\sim}{Q} = 6\pi\eta v r\,([C\,r x 0.33 \times 10^8] + 1) \qquad (3)$$

Equation (4) now states the conditions for which a protein ion has its charge, $\underset{\sim}{Q}$, proportional to its mobility,

$$\underset{\sim}{Q} = v\,(C' + C''), \qquad (4)$$

C′ and C″ being constants.

Making certain assumptions which are enumerated below equation (4) predicts the following rule:

In solutions of the same ionic strength, the electric mobility of the same protein at different hydrogen ion activities should be directly proportional to the number of hydrogen (hydroxyl) ions bound.

This statement includes the following assumptions:

(1) Complete dissociation of the protein salts or a constant fraction dissociated at different hydrogen ion activities.

(2) The hydrogen ions bound act as if they were at or very close to the surface or to the center and uniformly distributed.

(3) η and r do not change with pH.

(4) The reaction of the protein with ions other than the H^+ (OH^-) ion is negligible.

(5) Only uni-univalent electrolytes are considered.

(6) D and η of the medium can be used for their unknown values in the double layer, the effect of salts on D being unconsidered.

With these assumptions in mind examine the smooth curves in Figs. 1 and 2. These smooth curves are not a "best" curve but the titration curve of the proteins in a region where the activity coefficient of HCl is very nearly 1.00. Evidently, within the limits of error, the rule just stated is approximately followed and in the range investigated the electric mobility is proportional to the acid (base) bound.

According to Svedberg and Nichols[9] the egg albumin molecule under the foregoing conditions is spherical and has a molecular weight of about 35,000. At pH = 4.0, by thermodynamic methods, $\underset{\sim}{Q} = 25 \times 10^{-10}$ E. S. U. approximately, whereas using our approximation $\underset{\sim}{Q} = 15 \times 10^{-10}$ E. S. U. approximately. The general agreement as far as changes in v with pH is indicative of the underlying soundness of the theory and justifies theory and experiments seeking a second approximation. It is possible that the fact that the titration was done in HCl and the mobilities measured in acetate buffers may account for part of the difference between theory and experiment; or more likely the factor 6π is too small because of the distortion of the double layer. Using an empirical equation of the form of equation 3, the acid combining powers of Bence Jones protein, R-phycocyan and R-phycoerthyrin from the mobility data of Tiselius have been calculated and await experimental test.

Gelatin and Deaminized Gelatin

The rule that mobilities are proportional to the number of hydrogen (hydroxyl) ions combined with a protein in solutions of the same ionic strength has been tested in another way. Hitchcock[10] showed that deaminized gelatin adsorbed on collodion particles had an isoelectric point at about pH 4.0, and that acid was bound by the deaminized protein. In Fig. 3 is plotted the titration curve for "Cooper's Gelatin" and for the same gelatin deaminized by acetic acid and sodium nitrate. As before, the smooth curves, I and II, are the titration curves for gelatin and deaminized gelatin respectively. The closed circles which follow Curve I are the electric mobilities of quartz particles covered with gelatine in

N/150 acetate buffers. Curve I indicates that for gelatin itself, in solutions of the same ionic strength, the mobilities are proportional to the number of hydrogen (hydroxyl) ions combined. Let us assume (1) that after deaminization the average radii of cuvature of the surface of the deaminized gelatine is not appreciably changed by the loss of the amino groups; (2) that the dissociation of the deaminized gelatin salt in the range of pH studied is the same as for the gelatin itself; (3) that the type of adsorption of both gelatin and deaminized gelatin by quartz particles is the same, and that it represents a mean value of adsorption for a polydisperse system; (4) that the effective "molecular weight" is unchanged.

Under these conditions, all of which are reasonable, there should be obtained the following relationship:

$$\frac{\text{Combined } (\mp \text{H}^+) \text{ gelatin}}{\text{Combined } (\mp \text{H}^+) \text{ deaminized gelatin}} = \frac{\text{Mobility gelatin}}{\text{Mobility deaminized gelatin}}$$

That is, in the same buffer, the ratio of acid (base) bound for the two proteins should be equal to the ratio of their mobilities. That this is true experimentally is shown beautifully by the open circles plotted in Fig. 3 along Curve II. These open circles are the mobility values of deaminized gelatin and, as predicted by theory, they fall along the smooth titration curve of deaminized gelatin.

Casein

Proteins like the albumins are soluble in the region of the isoelectric point. For this reason the treatment of the relationship between combined

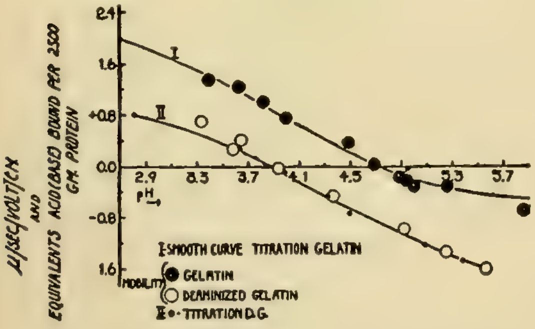

FIG. 3. In acetate buffer solutions of the same ionic strength, the ratio of the number of mols of hydrogen (hydroxyl) ions bound by gelatin and deaminized gelatin at a given pH is equal to the ratio of their mobilities.

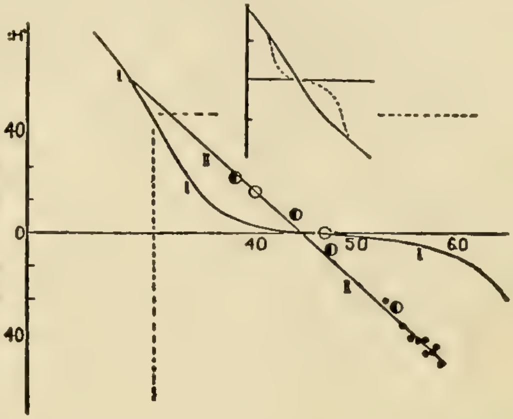

FIG. 4. The open circles (Loeb) and our data (half-closed circles) for the mobility of casein indicate that molecules of casein are highly charged on both sides of the isoelectric point. Calculations of the base bound (closed circles) by casein lead to the postulation of a smooth curve of the sort passing through the closed circles going through the isoelectric point in a linear fashion as indicated in the figure and agreeing in slope with the mobilities. The inset gives a clear picture of the usual "titration" curve (dotted line) and the titration curve here postulated (smooth curve).

acid and mobility has been uncomplicated by the insolubility exhibited by a protein like casein in the region of its isoelectric point. Fig. 4, Curve I-I-I, shows the "titration" curve of casein as ordinarily plotted. The flat portions of the curve are in the zone of a heterogeneous system. Loeb[11], on the other hand, pointed out that casein particles are highly charged on either side of the isoelectric point. The slope of the v-pH curve is large and corresponds to those for the other proteins just discussed. Curve I-I-I in the figure represents acid bound for "total casein" rather than for unit weight of protein dissolved. A serious discrepancy between our approximation and the relationship between combining power and mobility has been removed in the following simple fashion. Data in the literature have been recalculated so that values of hydrogen (hydroxyl) ion bound per unit weight of casein dissolved have been obtained. A straight line drawn through the mobility data for casein fits the new titration curve for dissolved casein reasonably well (Curve II-II-I). The slope of the titration curve of casein so plotted, agrees with the slope of the electric mobilities (plotted as before) of casein obtained by Loeb and by us ($\mu = 0.005$) in this region and meets the other portions of the curve in a reasonable fashion. These data point to the validity of our rule in the case of casein, and indicate a rational basis for the plotting of

titration curves in heterogeneous systems. In this instance, that of an insoluble protein, the mobility data give a much better index of the change in charge or ionization with pH than does the "titration" curve, *unless* the titration curve is plotted as combined acid per unit weight of protein, and the *dissociation* of the protein is known. The inset in Fig. 4 perhaps gives a clearer notion of the titration curve of casein as here postulated.

Insulin: Amorphous and Crystalline

In collaboration with Wintersteiner[12] the writer has compared the electric mobilities of adsorbed (amorphous) insulin and insulin crystals. From measurements of v on adsorbed and amorphous insulin particles. (Fig. 5A) the isoelectric point is at pH 5.35 in M/30 acetate buffers in agreement with somewhat similar data obtained by Howitt and Prideaux[13]. Compare the values of v obtained for *crystalline* surfaces of insulin (Fig. 5B, Curve II) with the *amphorous* surface (Curve I). It is reasonable to suppose that the differences in v obtained for amorphous and crystalline surface depend upon the changes in orientation of polar groups incidental to the formation of the lattice of the protein. This is supported by the fact that when sufficient dissolved protein is present to form a complete protein film of adsorbed protein on the crystal, the crystal surface acquires the electrokinetic properties of the amorphous particle or quartz particle covered with a protein film.

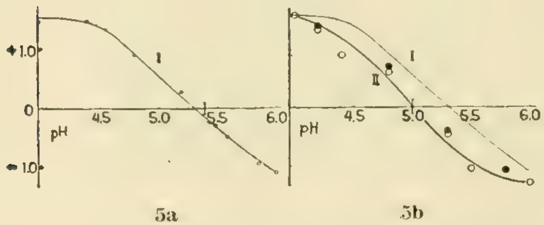

FIG. 5, A. The electric mobility of quartz particles covered with insulin in M/30 acetate buffers. The isoelectric point is between pH 5.30 and pH 5.35. The same data were obtained with particles of amorphous insulin. The ordinate units are in μ per second per volt per cm.

FIG. 5, B. The smooth curve (Curve I) gives the electric mobilities of adsorbed or of amorphous insulin. The lower curve (Curve II) gives the mobilities of insulin crystals or crystal fragments in the same medium. Curve II has been roughly fitted to the open circles (mobilities of crystals suspended in M/30 acetate buffer). For significance of the open and solid circles consult the text. The ordinate units are in μ per second per volt per cm.

Effect of Uni-univalent Salts

Since the study of proteins by the moving boundary method is necessarily carried out in the presence of a considerable concentration of salt, it is difficult to obtain the effect of increasing salt concentration on v by this method. Our information at present comes from surfaces of protein studied as the protein particle itself or adsorbed protein. Extensive studies of the effect of univalent ions on v have been made by Loeb[14], the pH being held nearly constant. The measurements of pH in these experiments were usually made in the absence of salt. For this reason a slight error is introduced into the values of pH given, the salt error increasing with the valence of the ions. Collodion particles, covered with a film of egg albumin, denatured egg albumin particles, casein and other proteins were among those investigated. The results of these numerous experiments may be stated briefly as follows: Small amounts of added salt, the pH held approximately constant, did not change v appreciably. Further addition of salt diminished v without the initial maximum in the v-c curve observed usually for inert surfaces, a limiting value for v, apparently not equal to zero, being approached. This is readily understood on the basis of the following reasoning.

Dissolved proteins and protein surfaces, like gelatin and egg albumin, differ in behavior in several important ways from "inert" surfaces. The charge of proteins in the absence of salts seems to depend mainly upon the pH, for at any given pH a certain number of hydrogen ions over a time average are attached to the protein molecule. In the special case under discussion, of a uni-univalent salt not shifting the isoelectric point[15], we can first for simplicity consider v to depend only upon κ, if the pH is fixed. The significance of this result is evident when we consider the Debye-Henry approximation, for the potential, ζ, at the surface (equation 2),

$$\zeta = \frac{Q}{Dr\ (\kappa r + 1)}.$$

Since v, is proportional to ζ, it will depend only upon κ if all other terms are considered constant, giving,

$$v = v\ (\kappa)\ pH = \text{const.}$$

By assuming that Q remains constant we do not by any means imply that no change in Q occurs incidental to changes in κ. It is merely postulated that the change in v with Q due to κ varying is very small compared with the change in v due to

explicit variation of κ. Addition of salt, under these conditions, then, should cause only a diminution in v without a maximum in the curve. We have used out empirical form of equation

$$\zeta = \frac{\underset{\sim}{Q}}{Dr\ (\kappa r + 2.4)}, \qquad (2a)$$

or

$$\zeta = \frac{\underset{\sim}{Q}}{Dr\ f(\kappa r)}$$

to plot in Fig. 6, by evaluating $f(\kappa r)$ for $r = 4 \times 10^{-7}$ cm. and various values of v for gelatin, the theoretical form of the v-c curves. Note the following points of interest in these curves.

The curves should in reality not cut the ordinate at c = O, for, in order to fix $\underset{\sim}{Q}$, a certain

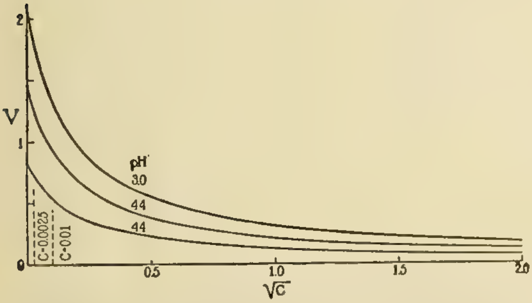

FIG. 6. Theoretical vm-c curves for gelatin at different values of pH, based upon single values given in Fig. 2. The two short vertical dotted lines at the lower left corner show the limits of extrapolation when the pH is sufficiently low to have appreciable amounts of acid present.

amount of acid must be present even though the concentration of protein and of salt is vanishingly small. In other words v is always measured in the presence of finite value of κ which is given for strong acids by the concentration, and not by the mean activity, of the acid. The dotted lines indicate, for example, the limiting position of the ordinate for c = 0.0025 M, and c = 0.01 M.

In more concentrated salt solutions the validity of equation 2 decreases; however, the curves indicate that v should be still quite large even in 4 M salt solutions. Technical difficulties at present prevent measurements of v in salts of this concentration; but values of the proper magnitude[16] have been observed by Hitchcock in M/10 acetate buffers for gelatin and by the author for serum proteins in solutions where c was equivalent to M/7. It would be most desirable to devise methods to discover if the available form of the theory is confirmed in that the prediction, v > O, is confirmed in concentrated salt solutions.

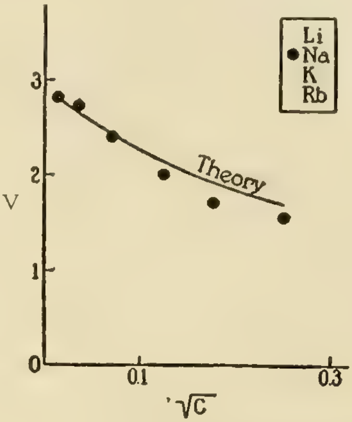

FIG. 7. Data of Loeb on particles of denatured egg albumin in 0.0002 M NaOH. Loeb found no important difference in the effect of the alkali halides. The smooth curve is calculated by means of the theory here proposed, based upon the highest value of v found by Loeb.

In Fig. 7 are values of v (Loeb) for denatured egg albumin particles in M/5000 NaOH. At this pH, in the absence of salt, v is rather high, 2.8 μ per sec. The smooth curve is the theoretical curve calculated by means of equation (2a) taking $r = 2.2 \times 10^{-7}$ cm. and making the usual assumptions in regard to η and D. It is noteworthy that the course of the theoretical and experimental curves are almost identical. Fig. 8 gives the results of similar experiments and calculations for egg albumin in acetate buffers, a correction being needed for the shift in the isoelectric point with variation in κ. Similar results were obtained for egg albumin in HCl and for gelatin in acetate buffers. To summarize: by assuming that the

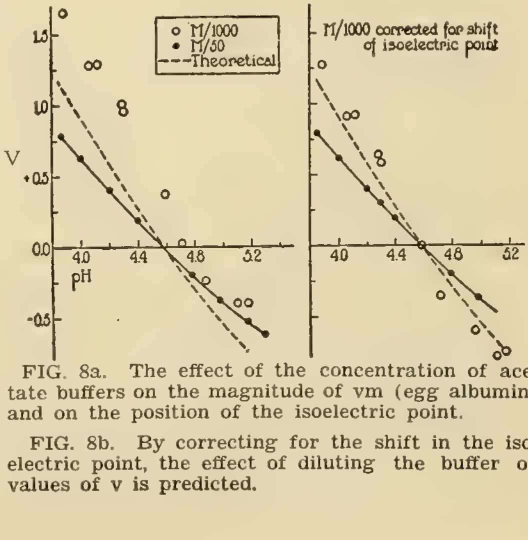

FIG. 8a. The effect of the concentration of acetate buffers on the magnitude of vm (egg albumin) and on the position of the isoelectric point.

FIG. 8b. By correcting for the shift in the isoelectric point, the effect of diluting the buffer on values of v is predicted.

electric charge of proteins is primarily determined by the hydrogen ion activity of the medium and by making corrections when necessary for shift of the isoelectric point, it is possible to derive a simple relationship between v and the concentration of uni-univalent electrolytes. This relationship, that v depends upon κ in a way which indicates that the decrease in the electric mobility of a protein ion with increasing concentration of a simple salt can well be understood by a diminution in the potential rather than in the charge, permits treatment of an electric mobility of an ion whose charge is given by a time average similar to that of an ion the charge of which is fixed.

Effect of Polyvalent Ions

Loeb[17], in particular, has investigated the effects of polyvalent ions on protein surfaces. In general, he found that ions of the same sign as

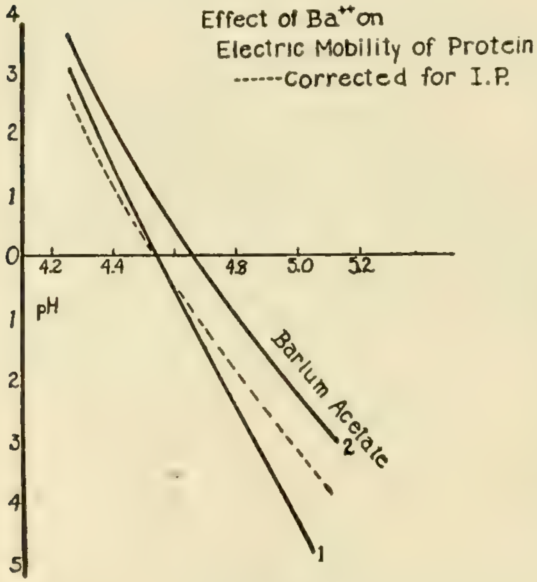

FIG. 9. Curves I and II are for egg albumin in Na— and Ba— acetate buffers. Note the shift in the isoelectric point and the change in shape of the curves. The dotted curve is Curve II corrected for the shift in the isoelectric point. (Data of Tiselius.)

that of the protein had effects more or less similar to the addition of univalent ions. Addition of polyvalent ions of opposite sign resulted in the reduction of the ζ-potential to zero and sign reversal. A consequence of this is that the isoelectric point of proteins should be shifted more by polyvalent than by univalent ions. That this is true for native proteins has been found by Tiselius[18] who studied the moving boundary of egg albumin and phycoerythrin in barium acetate buffers. This isoelectric point of egg albumin was shifted to the alkaline side; the barium ions probably reduce the net charge by reacting with the negatively charged albumin ions so that the isoelectric point is reached more quickly. The slight increase of v on the acid side is perhaps better understood if correction is made for the shift in the isoelectric point. If this is done (dotted line Fig. 9), note[19] that in accord with the viewpoint of Loeb there is hardly any effect of Ba^{++} on the acid side of the isoelectric point, but a noticeable change on the alkaline side. The ionic strength of M/50 barium acetate is slightly greater than that of M/50 sodium acetate. Part of the diminution in v (corrected) on the acid side may be due to this difference. Tiselius and also Koenig and Pauli have performed some experiments in unbuffered solutions.

Effects of Alcohol

Electrophoretic Velocity and Field Strength

The simple characterization of particles by measurements of the electrophoretic velocity, V, depends upon the fact that V is proportional to the field strength, X, as is evident from equation (1)

$$V = C \frac{\zeta D}{\eta} X = C' X$$

This linear relationship has been found with few exceptions. Thus Ettisch and Zwanzig[20] interpret their data on streaming potentials as indicative of a complicated relationship between V and X, particularly in alcoholic solutions, V increasing and reaching a limit with increase of X. Also Köhler[21] has reported that the volume velocity in electro-osmosis is not proportional to X. Daniel has made a careful study of the electrophoresis of gelatin, gliadin, egg albumin surfaces in various alcohols. Typical data are given in Fig. 10

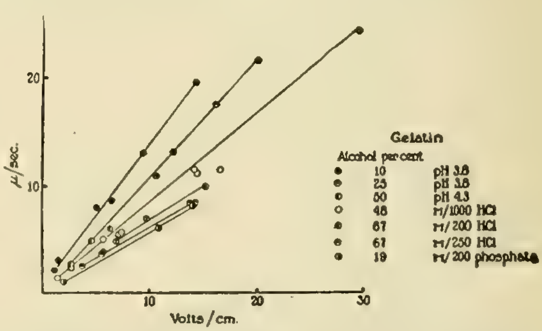

FIG. 10. The electrophoretic velocities of gelatin-covered quartz particles in media containing various percentages of ethyl alcohol are plotted against the field strength. In each medium the velocity is proportional to the field strength.

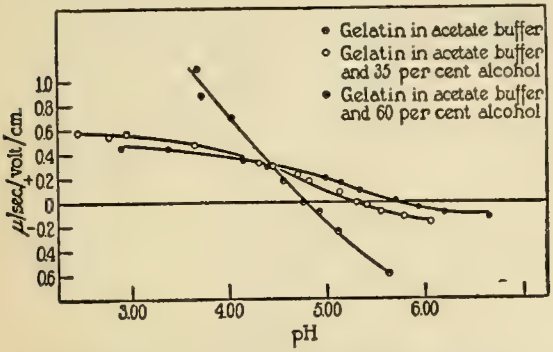

FIG. 11. The electrophoretic mobility of gelatin-covered quartz particles is plotted against the pH of the medium for media containing different percentages of ethyl alcohol. In the more acid regions NaCl-HCl mixtures were used in place of the acetate buffers.

for gelatin surfaces in ethyl alcohol. Between 2 and 30 volts per cm. V was proportional to X.

Electric Mobilities of Gelatin in Alcohol-Water Mixtures

Fig. 11 shows the mobilities of gelatin-coated quartz particles in N/150 sodium acetate buffer in 0 per cent, 35 per cent, and 60 per cent ethyl alcohol. Alcohol shifts the isoelectric point of the gelatin toward smaller hydrogen ion activities and lowers the maximum mobilities. This lowering combined with the shift in isoelectric point causes the curves to intersect. The diminution in v produced by alcohol is not a simple phenomenon. Alcohol changes at least both the dielectric constant and the viscosity of the medium and may also be expected to alter the electrokinetic potential[22].

Differences in the mobilities which were due to altered viscosity were eliminated by Daniel by calculating the mobility corrected for the viscosity of the medium,

$$\text{corrected mobility} = v\eta/\eta_0$$

This quantity has the significance that the differences between curves of corrected mobilities should be due to changes in the dielectric constant alone, the values being in some ways more representative of the effect of the alcohol itself on v.

The data in Fig. 11 and 11a give the mobilities uncorrected for η. In Table I there are compared values obtained from smooth curves of v and v η/η_0 for equal charge (as determined by the amount of acid bound). The large differences in v disappear almost completely when the correction for η is applied, only a slight decrease taking place as the alcohol concentration increases. This result is similar to Walden's results for ions.

Mobility, Titration Curve and Charge

By comparing the mobilities in the different media (differing in dielectric constant and viscosity) it is possible to test to some extent the applicability of the viscosity and the dielectric constant of the bulk of the medium to the electro-

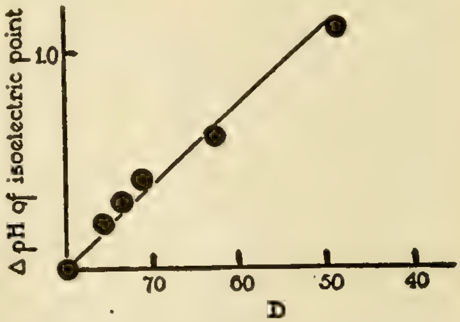

FIG. 11a. Above, the change in pH of the isoelectric point of gelatin, caused by ethyl alcohol, is plotted against the volumes per cent. alcohol in the solution. Below, the same data are replotted as change in pH of the isoelectric point against dielectric constant of the solution.

phoresis equation for charge, equation (3). The simplest means if doing this is to calculate charge from mobility by means of equation (3), using the viscosity and dielectric constant of the bulk of the medium. Note that a suitable correc-

TABLE I.

v			$v\ \eta/\eta_0$		
0 per cent alcohol	35 per cent.	60 per cent.	0 per cent.	35 per cent.	60 per cent.
0.20	0.08	0.06	0.20	0.20	0.16
0.40	0.15	0.10	0.40	0.40	0.30
0.60	0.21	0.15	0.60	0.54	0.42
0.80	0.25	0.19	0.80	0.67	0.54

The figures in each horizontal row are for pH's of equal charge as determined by the titration curves.

tion must be made in κ for the lowered dielectric constant.

Since

$$\kappa = \sqrt{\frac{4\pi e}{D k T} \sum_{1}^{s} n_i z_i^2}$$

If the acid bound (measured directly) is, in different media, in the same ratio as the charge calculated from the mobility by equation (3), then within the limits of the experimental error equation (3) may be used to predict changes in charge, using the viscosity and dielectric constant of the bulk of the medium. (See previous sections for other assumptions).

Fig. 12 shows the agreement between Q from equation (3) and titration curves in the middle pH region for 0 per cent and 35 per cent alcohol. This graph was made by drawing the 0 per cent alcohol titration curve and charge points to scales which made them coincide and then drawing the 35 per cent titration curve and charge points to the same scales. All the charge points calculated from mobilities determined in acetate buffer fall very well onto the titration curve.

All of the data for acetate buffers and NaCl-HOl mixtures from pH 2 to pH 7 have been plotted in Fig. 13, titration curves in the upper half, mobility curves in the lower. The titration curves of gelatine in 0 per cent. and 35 per cent. alcohol have been compared by Daniel from pH 2 to pH 10. The curves are very much of the same shape, the isoelectric point being shifted to a higher pH, the curves converging at the limits. The experiments of Daniel comprise one of the most striking examples of the fundamental

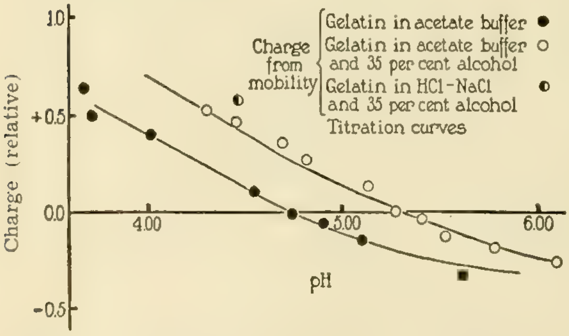

FIG. 12. The full circles show the charge of gelatin calculated from the mobility of gelatin-covered quartz particles in acetate buffer. The open circles show the charge calculated from the mobility in acetate buffer and 35 per cent. ethyl alcohol. The lines are titration curves of gelatin in 0 per cent. and in 35 per cent. ethyl alcohol. The figure is limited to a range fairly close to the isoelectric point.

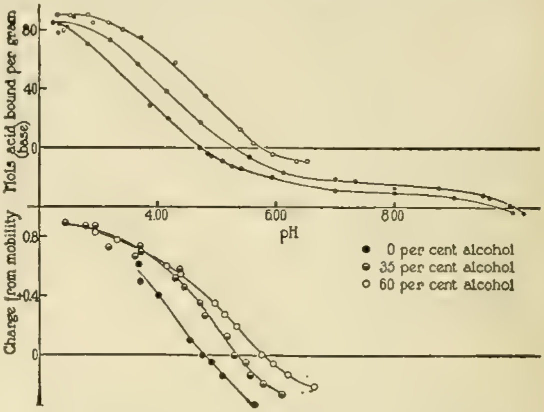

FIG. 13. The upper curves are titration curves of gelatin in 0 per cent., 35 per cent., and 60 per cent. ethyl alcohol. The lower curves are the charge curves calculated from the mobility of gelatin-covered quartz particles in 0 per cent., 35 per cent., and 60 per cent. ethyl alcohol, the circles being experimental points.

validity of the assumptions of the Smoluchowski theory of the double layer and of the usefulness of the modern theory of electrolytes in dealing with electrokinetic phenomena.

Mechanism of Adsorption of Protein

The fact that not only the isoelectric points but also the electric mobilities of quartz particles covered with serum albumin or egg albumin are very nearly identical with the values of mobility found for the respective dissolved protein indicates that practically *all* the polar groups of the protein molecules are available even after adsorption has occurred. To demonstrate this let us suppose that one of the hydrogen ions is lost incidental to the adsorption reaction. Near the isoelectric point one H^+ added to each protein molecule gives it a mobility of about 0.10μ per sec. per volt per cm. This very small change can conceivably have occurred in the case of egg albumin, but it is not evident for serum albumin. Since the higher mobilities are practically identical, no change greater than the loss of one H^+ is probable. In other words, adsorption of a large molecule such as a protein permits practically the full activity of the polar groups to be made manifest in spite of the adsorption. Fig. 14 illustrates schematically what can conceivably occur, the reaction between quartz and protein taking place possibly without appreciable loss of charge. Theoretically a change in the mobility of the protein-covered quartz particles could have occurred also for the following reason. If we utilize the theory of the rigid double

layer to give a qualitative picture of what occurs, if d is the thickness of the double layer,

$$d = \frac{1}{\kappa} \quad \frac{\kappa r}{\kappa r + 1}$$

Now r represents more strictly the effective radii of curvature of all points on the surface of the protein molecules or of the quartz particles. To have the protein-covered quartz particles possess mobilities identical with those of individual molecules, it seems necessary that (κr) remains unchanged, each molecule on adsorption taking effectively its own (κr) along with it; for $v = f$ $(\kappa r$ and (κr) would vary sufficiently to affect v if any important change in r occurred. In calculating Q for protein-covered quartz particles it is necessary to know the radius of the spherical molecules themselves. The bulk radius of the microscopically visible quartz particles is then probably not the mean radius of curvature of the surface. The calculation of Q for blood cells, bacteria, and other microscopically visible particles will always be complicated by the difficulty of ascertaining the effective values of r. If the mobilities are independent of size and shape of the particles, however, and if comparative measurements are made in solutions of the same ionic strength and species, the mobilities are proportional to the charges and a very good idea of the charge can be obtained by means of equation (2a). The reasoning in regard to (κr) for surfaces in general leads to the establishment of criteria which are necessary for the complete identity of surfaces. It is necessary that not only the chemical (atomic) structures of two surfaces be identical and not only (κr) but also κ and r for each. Identical surface density of charge does not mean identity of surface properties. To illustrate this point imagine a protein molecule having $r = 2.17 \times 10^{-7}$ cm. and a smooth surface, growing larger and larger to say, $r = 1 \times 10^{-4}$ cm., its charge density remaining constant, and the surface still retaining its smoothness; for $\kappa = 0.33 \times 10^7$, utilizing the theory of Henry it can be readily shown that the mobility of the larger particle should be very much greater. Conversely, if the ζ-potential of the two different surfaces is the same, the effective radii of curvature of the surfaces may be producing changes bringing chemically different substances to the same ζ-potential.

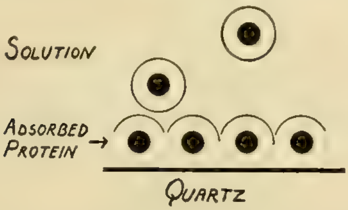

FIG. 14. Schema of proposed mechanism of adsorption of proteins like egg albumin and serum albumin. The protein molecule (central black filled circle) and the outer layer of the double layer (outer circle) are represented without their charges for convenience. Four molecules are adsorbed. Two are free in solution. According to the mechanism here postulated, (1) the adsorbed protein molecules adsorbed have their radii or equivalent radii unchanged. They do not "lie flat" at the interface. (2) The effective thickness of the ion atmosphere about each molecule at the interface is the same thickness as that found for molecules in solution. (3) The available charges are practically the same. (4) The protein molecules determine the nature of the ion atmosphere, the quartz surface playing a negligible role at the interface.

Activity of Adsorbed Invertase

The fact that adsorption need not involve certain properties of the polar groups of large molecules simplifies the explanation of a phenomenon observed by Nelson and Griffin[23]. These investigators found that, under certain circumstances, *adsorbed* invertase did not lose a significant portion of its enzymatic activity. This is in complete harmony with the facts discovered relative to protein adsorption. It is easily conceivable that enzymes that are protein-like in nature could be adsorbed or be active at an inert or living surface without diminishing either the number of the enzymatically active groups or the activities of these groups qualitatively and quantitatively.

The Validity of the Mass Law

The fact that the same values have been obtained for mobilities of molecules dissolved in a homogeneous system and of molecules existing at a phase boundary indicates that the mechanism of adsorption *per se* need not change the properties of the reactive groups. It could have been anticipated that the forces at a phase boundary would have disturbed the dissociation equilibria, yielding different apparent dissociation constants. This has not occurred. This idea has been developed by Michaelis[24] in connection with enzymatic behavior of invertase.

The Action of Immune Sera

Shibley[25] has shown that certain bacteria treated with immune sera have electrophoretic velocities practically equal to that of serum globulin particles. The reaction of the bacteria with specific groups belonging to serum globulin can occur without disturbing the amphoteric proper-

ties of the globulin as the simpler models here studied indicate.

Further Experimentation

The difficulties of the moving boundary method, in particular the fact that it cannot be used for proteins in dilute salt solutions justifies the experimental extension of data of the type obtainable by the microscopic method employed here. This method can be used over practically the entire pH range usually studied with solutions from infinite dilution to solutions having the conductance of physiological salt solutions. By observance of the principle of having ionic strength and ionic types identical, the properties of the proteins possibly dependent upon their charge can readily be investigated and classified. This has been done for optical rotation elsewhere.

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Discussion

Dr. Cohen: Is there any information that the adsorbed protein is in crystalline form as against amorphous?

Dr. Abramson: Yes, there is some information. As noted, I have studied particles of crystalline insulin, which is highly insoluble at its iso-electric point. I was able to study (1) adsorbed insulin on both sides of the iso-electric point, (2) amorphous insulin, and, near the iso-electric point, (3) crystalline insulin. The adsorbed insulin and the amorphous insulin had the same mobility-pH curves, whereas the particles of crystalline insulin had a different iso-electric point, but approached that of the adsorbed and amorphous insulin when sufficient quantity of the latter was present to cover the crystals themselves. The insulin crystals, thus covered, act just like a quartz particle with adsorbed insulin.

Dr. Bates: Since addition of salt to a solution of gelatin in HCl, changes the pH of the solution, will you tell the manner in which this fact is taken into account?

Dr. Abramson: I either measured pH, or corrected Loeb's data so that the activity coefficients of the salts would give the correct value of pH.

Dr. Müller: In investigating the change of the mobility with κ don't you have to know the radius of the particles?

Dr. Abramson: I assumed that the mean radius of the curvature, at every point on the particles, is unaffected by a change in κ.

Dr. Chen: Does the preparation of amorphous insulin involve processes which might have modified its identity with the crystalline insulin? Do you have data, such as solubility, to show that the amorphous insulin and the crystalline insulin are the same? Can the amorphous insulin be crystallized?

Dr. Abramson: The amorphous insulin was prepared by merely changing the pH. It was used immediately. The amorphous insulin can be crystallized.

Dr. Blinks: Have you found any cases where the iso-electric point does change simply by adsorption?

Dr. Abramson: Not as yet, but I expect that it will be found, since denaturation occurs when

serum globulins are "adsorbed" from immune serum.

Dr. Cohen: Will you amplify your remarks about the iso-electric point of ampholytes with reference to dissolved amino acids and their crystals?

Dr. Abramson: Historically the term isoelectric point was first used to designate a reference concentration at which the electric mobility of a particle of any sort was zero. It later became of importance in connection with *dissolved* ampholytes. On the basis of usage, therefore, a *crystal* of an amino acid, has an isoelectric point, as well as the dissolved amino acid. In the case of a particle of a crystal suspended in an acid, the isoelectric point of the crystal is the pH where the electric mobility is zero or, more formally where,

$$\frac{1}{\mathbf{T}}\int_{0}^{T} dt \left[\Sigma\, n\, (\mathbf{e}) + \Sigma\, n\, (-\mathbf{e}) \right] = 0,$$

$$T >> \tau$$

that is where time average of the total sum of all positive and negative ions in residence at the surface is rezo (τ is the time of residence of the ion having the longest period at the surface). This definition is quite general. The isoelectric point of a monobasic, monoacidic, dissolved amino acid, A, is the pH where,

$$[A^{+}] = [A^{-}] \text{ ,}$$

so that, in accordance with the preceding general equation, the time average of the net charge is zero, and, consequently, our general definition is applicable to both particulate and dissolved amino acid. Investigations on the isoelectric point of crystals of relatively insoluble amino acids have shown that the particles' isoelectric point bears no simple relation to that of the dissolved substance.

Dr. Cohen: Would you define an amorphous material?

Dr. Müller: There is no sharp limit between the amorphous and crystalline state structure. Even liquids have to a certain degree a crystallographic structure, and, according to Zeicky's theory of the mosaic structure of crystals, every crystal has amorphous regions. Usually the surface of a crystal has a distorted lattice.

AGGLUTINATION

STUART MUDD

Three principle factors have come to be recognized as governing the stability of colloidal dispersions, i. e., the electrokinetic potential difference of the individual particles, the solvation of the particles, and the force of cohesion between the particles when in contact. In this lecture the relation of these factors to the general problem of colloidal stability is discussed, and there is suggested a simpler treatment of colloidal stability in terms of (1) the probability of collision of particles, and (2) the probability of cohesion of collided particles.

Agglutination phenomena of bacterial and other cells represent special cases of colloidal aggregation. Instances already studied show that bacterial suspensions range from those owing their stability solely or principally to electrokinetic p.d. through intermediate cases to those owing their stability solely to hydration or to lack of cohesiveness. Examination of these instances of bacterial agglutination may serve to illustrate and even to extend the principles of general colloidal aggregation.

*The Stability of Dispersions of Colloidal Particles in Water and Aqueous Solutions**

There are two necessary conditions for the existence of a stable dispersion of colloidal particles in an aqueous medium. The first is that the mass and size of the particles be small enough so that they remain suspended for the period under consideration against the force of gravity[2]. The second is that some factor must operate to prevent the aggregation of the particles to form larger masses which would no longer remain suspended.**

Given a dispersion of particles in water whose mass and size are sufficiently small so that settling is slow, three general factors must be considered as determining whether or not appreciable aggregation of the particles takes place within a given period of time. The first condition requisite to the aggregation of particles is that their Brownian motion must bring them into contact with one another. Therefore the first general factor which must be considered is the rate at which Brownian motion tends to bring this about. The major experimental factors affecting this rate are the concentration, or number of particles per unit volume of the dispersion, the mass and size of the particles, the viscosity and the temperature of the suspension[3]. Study of the effect of variation in these factors upon the rate of agglutination of bacteria is of interest[4]. However the observations of the agglutination of bacteria which are of direct importance in bacteriology and immunology are made under fairly constant conditions, so far as these factors are concerned. These usual conditions are such that the expectation of collision due to Brownian motion in the absence of repelling force is sufficient to cause rapid, complete aggregation of the bacteria if each opportunity for collision results in contact of the particles, and each contact results in cohesion. For these two reasons further discussion of the effect of variation in factors which affect the rate of opportunity for collision due to Brownian motion is unnecessary for the purposes of the present lecture. Discussion may be found in comprehensive treatises on colloid chemistry, and the most recent elaboration of the kinetics of rapid coagulation has been given by Dr. Müller in the preceding lecture of this symposium. Northrop has discussed these factors in relation to bacterial agglutination[5].

The second general factor which must be considered as determining whether or not appreciable aggregation of colloidal particles will take place within a given length of time is the probability of contact when opportunity for collision is provided in consequence of Brownian motion. It is clear that such contact must occur unless prevented by some repelling force acting between two particles tending to collide in virtue of their Brownian motion. If this repelling force is sufficient to overcome the momentum of the particles contact will not occur. There must obviously be a value of any such repelling force at which it just balances the momentum of the particles. This may be called the critical value of the force in any case.

The critical value described is strictly applicable only to a single pair of particles tending to collide under given conditions. In any system of dispersed particles, there is a statistical distribution of velocities due to Brownian motion[6]. Therefore a given repelling force may prevent certain collisions and not others in any given case. The critical value of the repelling force for a system of dispersed particles is therefore the value which is just sufficient to prevent a sufficient majority of contacts from taking place when opportunity is offered for collision.

* The theoretical discussion which follows is essentially that of Mudd, Nugent and Bullock[1].

** The mass and specific gravity of bacteria is such that if no appreciable aggregation occurs, the amount of settling which takes place in eighteen hours is sufficiently small to be neglected. For this length of time, therefore, bacterial suspensions may be treated as suspensions of colloidal particles.

The third general factor is the probability of cohesion after contact has been made. The interface between a dispersed particle and its dispersion medium is the seat of free surface energy equal to the free interfacial energy per unit area multiplied by the surface area of the particle. Contact of two particles results in a decrease in free surface energy* equal to twice the area of contact of the particles, times the interfacial tension, or:

$$\Delta F = 2\ S.\gamma AB$$

where ΔF is the decrease in free surface energy, S is the area of contact of the two particles in contact and γ AB is the free interfacial energy per unit area.

Following Harkins'[7] general treatment of work of cohesion, it is apparent that the work of cohesion between two such particles in contact is measured by the free energy increase necessarily attendant upon their separation under ideal conditions,*
or:

$$W\ c = 2\ S.\gamma\ AB$$

where Wc is the "work of cohesion" and the other symbols have the same significance as before.

In order to cause separation after contact has been made, the dispersive forces* must provide a minimum energy equal to Wc. If Wc is greater than the energy provided by the dispersive forces the particles will cohere after contact. If it is less the particles will separate again after collision. Obviously here too, as in the case of the repulsive force, there must be a value of Wc which will just balance the dispersing tendency in any case. This may be called the critical value of Wc.

As in the case of the concept of a critical repelling force, so also in the case of the concept of a critical work of cohesion, it is important to bear in mind the statistical distribution of kinetic energies. The Brownian motion impulses tending to separate particles in contact vary in magnitude in a statistical manner. The critical work of cohesion for a system of dispersed particles is therefore such that a sufficient majority of the impulses tending to separate particles in contact fail to do so.

It is apparent from the foregoing considerations that if the repelling force is greater than its critical value, a dispersion will be stable. The same is true if the work of cohesion is less than its critical value.

The question of the variation of the work of cohesion merits particular discussion at this point, remembering that the present discussion applies to dispersions in aqueous media. The work of cohesion has been defined as equal to 2 S. γ AB. The variation of work of cohesion from case to case is therefore primarily a matter of variation in the free surface energy at the respective particle-dispersion medium interfaces. In general, in accord with Harkins[8], the more nearly similar the dispersion medium and the surface material of the particles, the lower the expected interfacial tension. In this connection, it is most important that certain colloidal particles have the property of associating themselves with large quantities of water from their dispersion medium[9]. It is not necessary at this point to discuss the possible mechanisms involved in the taking up of the water. It is highly probable that different mechanisms are operative in different cases[10]. It is however also highly probable that in some of these cases the combination of the particles with water results in a hydrous particle surface, which is much more similar to water in the Harkins' sense than the surface of a particle of the same substance in the anhydrous condition. In such cases the interfacial tension of the particles against their dispersion mediums is presumably lowered and along with it the work of cohesion of the particles. It would seem that the lowering of work of cohesion due to the hydration of the surface of dis-

* The free interfacial energy as considered here involves any effects due to the existence of an electrical double layer at the particle-dispersion medium interface. It is a composite result of the interaction of three sets of force fields, those of the particle molecules, those of the dispersion medium molecules and ions and that due to the existence of the double-layer. No assumption is necessary here, and none is made as to the equality of the interfacial tension at the micro-particle-dispersion medium interface referred to and the interfacial tension at a macro-interface between the dispersion medium and the material of which the particles are composed.

* In circumstances under which surface films have coalesced separation may be non-ideal and involve also work against viscosity.

* Exact definition of these dispersive forces is difficult or impossible. It seems, however, that at least three factors may be recognized in a qualitative way:

(1) The Brownian motion itself.

(2) Electrostatic repulsion. Hydrophilic colloids which are ionogenic at least owe part of their electrokinetic p. d. to ionization at fixed points on the particle surface. All of these points obviously can not be in contact. It is probable therefore that there is some residual electrostatic repulsion even between particles coherent over a part of their surfaces.

(3) The tendency of the water molecules to wet hydrophilic substances in the surfaces of the coherent particles may tend to force these coherent surfaces apart.

It is also possible that statistical fluctuations in the internal energy of the molecules of colloidal particles in contact must be taken into account in a complete treatment of dispersive forces. (In this general connection see Burk: J. Phys. Chem., 35, 2446 (1931)).

persed particles must be considered as a potential factor affecting the work of cohesion between them and thus the stability of dispersions of such particles in aqueous media.

The relationship of the foregoing material to the well known experimental facts with regard to the stability of colloidal solutions is fairly obvious. Colloidal particles dispersed in aqueous media, may be conveniently considered in two classes for purposes of discussion, as hydrophobic particles and hydrophilic particles. The first class have little or no affinity for water and the second a marked affinity.

In the case of dispersions of hydrophobic particles clear-cut experimental evidence[11] has shown that the necessary condition for their stability is that the electrokinetic potential difference at the surface of the particles exceed a certain limiting value known as the critical potential[12]*. When the electrokinetic potential falls below this value aggregation of the particles takes place. The electrokinetic potential difference between particles and their dispersion medium results from the existence of an electrical double layer at the surface of the particles. The particles are positively or negatively charged with respect to the medium depending, respectively, upon whether the positive or negative side of the electrical double layers is associated with the particles. It has been believed that the repelling action of similarly charged particles is responsible for the stability of suspensions of hydrophobic particles when the electrokinetic potential exceeds the critical value[13]. Dr. Müller has just given us a further interpretation of the nature of the stabilizing action of the electrical double layer.

In terms of the general working theory of suspension stability which has been presented, this electrical double layer is the force which if sufficient can prevent the contact of particles when opportunity for their collision is provided in virtue of their Brownian motion, and thus stabilize the dispersion.

Turning now to the question of the stability of hydrophilic colloidal particles, it is found that quite a different situation prevails. Kruyt and others working in his laboratory[14] have clearly demonstrated that the condition of hydration of certain hydrophilic colloidal particles must be considered as a stabilizing factor in dispersions of such particles in aqueous media. The first outstanding fact is that certain of the particles studied formed stable dispersions when their repelling force, as measured by their electrokinetic potential, was reduced to zero[15]. The addition of sufficient alcohol to such isoelectric dispersions caused them to precipitate. The alcohol in such cases is generally considered to act by dehydrating the particles. These facts indicate first that a stabilizing factor is active apart from electrokinetic potential difference, and secondly that this stabilizing factor results from the hydrated condition of the particles. It is apparent that this second stability factor is capable of stabilizing a dispersion of these particles in complete absence of a repelling force. It must therefore act by decreasing the work of cohesion of the particles below the critical value or by raising this critical value. Reasons for this stabilizing action associated with the hydrous condition of the particles then follow directly from the previous discussion. It was pointed out that increased surface hydration should accompany the union of hydrophilic particles with water of the dispersion medium. This increased surface hydration should cause the hydrated particles to have a much lower surface tension against the aqueous dispersion medium than would the same particles in a hypothetically anhydrous condition. Certain hydrous particles might well thus have very low surface tensions against aqueous media which in turn would cause them to have very low works of cohesion, even possibly below the critical value. In this way the hydrous condition of particles in certain cases could be a stability factor which could result in the stability of a suspension of such particles even when their electrokinetic potential was reduced to zero. Moreover the tendency of the water to wet the hydrophilic surfaces might promote dispersion and therefore necessitate a high critical value of the work of cohesion.

The mechanism outlined above is here offered as the one which is operative in the unquestioned stabilizing influence of the hydrous condition of the particles in hydrophilic suspensions. It is significant that the only dispersions of colloidol particles in aqueous media which are known to be stable in the absence of electrokinetic potential are those in which independent evidence points clearly to the hydrophilic nature of the particles.

It should be pointed out that the concept of variation in the work of cohesion with surface hydration applies to variation in the state of hydration of a particular surface. In passing from one surface to another, as in the deposition of a protective or sensitizing film, no such relationship necessarily exists. Surface A may be less hydrous than surface B and still have a lower work of cohesion. The point is that surface A for example presumably has a lower work of cohesion in a relatively hydrated state than in a relatively dehydrated state. In the sense of this lecture, changes in hydration of a particular surface such as may be brought about by the electrolyte con-

*The critical potential referred to is the "first" critical potential (12).

tent of the medium are considered as modifications of an existing surface rather than the formation of a new surface.

The critical potential as experimentally determined for a system of dispersed particles is the minimum electrokinetic potential compatible with a stable condition of the dispersion under the defining conditions. Suppose that the work of cohesion is sufficiently high so that practically every contact results in permanent coherence of particles. The experimentally determined critical potential will then be such that it is just sufficient to prevent a sufficient majority of contacts when opportunity for collision is offered due to Brownian motion. If the work of cohesion is somewhat lower, that is if an appreciable number of contacts result in redispersion, the repelling force would not have to be quite so large, that is it would not have to prevent as many contacts as before in order to maintain stability. In this second case, the experimentally observed critical potential would be somewhat lower than in the first.

Theoretically, therefore, in all cases in which the work of cohesion is insufficient to prevent all redispersion, experimentally determined critical potentials should decrease with decrease in work of cohesion. When the work of cohesion is sufficiently small the suspension will be stable even at zero electrokinetic potential.

Shibley[16] confirmed the critical potential value of Northrop and De Kruif[17] ($\pm$ 15 millivolts) with bacteria suspended in NaCl, $ZnSO_4$ and $CeCl_4$. In the presence of Na_2HPO_4, however, the same microorganisms had a much higher critical potential (—34.6 millivolts in one experiment). It is possible that the higher critical potential in the presence of Na_2HPO_4 was due to an increase in work of cohesion due to this particular salt.

Aggregation occurs when the electrokinetic potential difference is lower than its critical value in any system of dispersed particles, whose cohesive force is above its critical value. However the *rate* of aggregation within the critical potential zone varies with the residual p.d. upon the particles. One reason is that the lower the p.d. the greater the majority of total opportunities for collision which result in contact, with all opportunities for collision resulting in contact at the isoelectric point. Further, it has been suggested that residual potential difference is a factor aiding the redispersion of particles after contact, at least in some cases. In these cases, the greater the residual p.d., the greater the redispersion tendency and presumably the slower the rate of aggregation.

The relationship of stabilizing and sensitizing surface films to the general question of the stability of dispersions of colloidal particles in aqueous media is secondary to the factors which have been discussed. It will be treated in the section of the lecture dealing with the stability of suspensions of sensitized bacteria.

The Stability of Suspensions of Unsensitized Bacteria

The work of Northrop and De Kruif[17,18] has been of great importance in the development of the theory of bacterial agglutination. The stability of the suspensions of the two types of bacteria which they studied varied markedly and regularly with the salt content of the dispersion medium. With salt concentrations below 0.001 molar both types regularly agglutinated when their electrokinetic potential was reduced below $\pm$ 15 millivolts. Under these conditions $\pm$ 15 millivolts was the critical potential. When the total salt concentration was raised to 0.1 molar, the suspensions were stable when the electrokinetic potential was reduced to much smaller values than $\pm$ 15 millivolts, in some cases even when it was reduced to zero.

Shortly after the work of Northrop and De Kruif, Loeb[19] showed that the stability of gelatin solutions is influenced by salts in an exactly similar way, and further that the stability of suspensions of collodion particles coated with surface films of gelatin also showed the same type of behaviour. Loeb pointed out the similarity of his results of this type to those obtained by Northrop and De Kruif with bacteria. He concluded from his experimental work that the increased stability of the suspensions of protein-coated collodion particles in the higher salt concentrations was most probably due to increased affinity of their surfaces for water under these conditions. Later Oliver and Barnard[20] and Netter[21] also attributed the decrease in "cohesive force" of the surface of cells by salts to increased affinity of the surfaces for water. It would seem highly probable on this basis that the increased stability of the bacterial suspensions of Northrop and De Kruif may also have been due to an increase in the hydrous condition of the surfaces of the bacteria in the higher salt concentrations. This probability also follows from the theoretical considerations which have been presented in this paper.

Since the suspensions were stable in some cases even when the electrokinetic potential was reduced to zero, it follows that the work of cohesion of the bacteria must have been reduced below its critical value. It was shown that the most probable cause for the reduction of the work of cohesion between particles dispersed in aqueous media, is an increase in the hydrous condition of the particle surfaces. Changes in salt concentra-

tion are well known to affect the state of hydration of hydrophilic colloidal particles, and hence very probably to alter their state of surface hydration.

Northrop and De Kruif clearly recognized that a decrease in "cohesive force" must have taken place as between their bacteria in 0.001 and 0.1M salt solution. They devised and used an ingenious method for following changes in this value. They defined "cohesive force" by the values obtained by this method. There is some question as to whether the values obtained by them accurately expressed the value of the cohesive force as defined in this paper, because, for example, their method may well have involved work against viscosity in the separation of partially coalescent particles. Nevertheless, in any case, they definitely showed that a reduction in "cohesive force" as measured by their method always accompanied the phenomenon of suspension stability with electrokinetic potentials below ± 15 millivolts. This parallelism is convincing evidence that they were able to measure true cohesive force with sufficient accuracy to arrange their suspensions in the proper order with regard to their value, and that was the most important object of their "cohesive force" measurements.

Northrop and De Kruif further showed that if the salt concentration is increased well beyond 0.1 molar their bacterial suspensions again became unstable. There seems to be no question but that they were correct in attributing this to a "salting out" mechanism, that is to a dehydration and precipitation in the presence of a high salt concentration. The general conclusion from their results is that both electrokinetic potential difference and hydration are important factors in determining the stability of suspensions of the two types of bacteria studied by them, both being markedly affected by variations in the total salt content of the dispersing medium.

According to Northrop and De Kruif, the lowest concentrations of salts acted to affect the electrokinetic potential difference, medium concentrations to affect the "cohesive force" and still higher concentrations to affect the state of surface hydration. It appears that the effect of the intermediate concentrations on "cohesive force" may also be interpreted as resulting from an effect on the hydration affinity of the bacterial surfaces.

Bacteria do not all have surfaces of this type, however. We have studied by the interfacial technique[22] the relative ease of wetting by oil and water of the surfaces of a large number of different types of bacteria. It has been found in this way that acid-fast bacteria are in general more readily wet by oil than by water whereas non-acid-fast bacteria in general are much more readily wet by water.[23] It would be expected on this basis that stability relations of suspensions of acid-fast bacteria would resemble those for hydrophobic colloidal particles rather than those for hydrophilic particles.

In contrast to these acid-fast bacteria are certain strains of aflagellate intestinal bacteria which have been studied in the writer's laboratory. In their "smooth" form these bacteria have electrokinetic potentials so small as not to be with certainty measureable over a wide range of pH values and electrolyte concentrations.[24] See Table 1. Yet these bacteria without measurable p. d. are stable in suspension.

Table I.

The electrophoretic mobility of Bact. flexneri "smooth" and "rough" as a function of pH.

Buffer	pH	Smooth μ/sec/ volt/cm.	Rough μ/sec/ volt/cm.
Phosphate	7.1	0.1	3.4
"	6.6	0.0	3.9
"	6.0	0.0	2.9
Acetate	5.7	0.1	3.9
"	5.2	0.05	3.8
"	4.8	0.1	3.6
"	4.4	0.0	3.4
"	4.0	0.0	3.1
"	3.7	0.0	2.6
Phthalate	3.3	0.1	2.4
"	2.6	0.0	2.1

The general contention of Northrop and De Kruif was that the stability of their bacteria with very low electrokinetic potentials when the salt content was increased to above 0.1 molar, was that the increased concentration depressed the cohesive force of the bacteria. Since the abovementioned strains of bacteria form suspensions which are stable in distilled water or very dilute electrolyte, with electrokinetic potentials of zero to a few millivolts, it is apparent that an extension of the views of Northrop and De Kruif is necessary to account for the stability of these suspensions.

According to the theoretical conclusions of the present paper it appears necessary that this type of stability is due to an extremely low work of cohesion due to surface hydration which is probably in excess of that obtaining in the case of the bacteria of Northrop and De Kruif. It is at least quite definite that the primary stabilizing action of hydration is operative over a wider range of conditions in the case of the strains described here. Acid-fast bacteria and the type just described represent extreme types selected from a large number of bacteria studied over a period

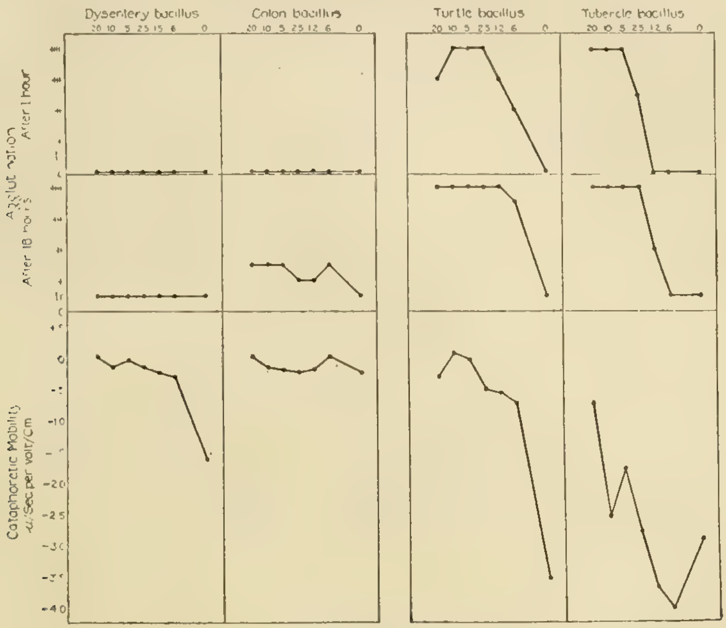

Fig. 1. Stability of hydrophilic bacilli and precipitation of hydrophobic bacilli in presence of acid. Washed bacteria suspended in solutions of HCl in distilled water. Abscissae, concentrations of HCl in millimols per liter. The hydrophilic dysentery and colon bacilli show little aggregation at any acidity. The hydrophobic turtle and avian tubercle bacilli show complete aggregation in acid concentrations which sufficiently reduce the electrokinetic p. d. To obtain electrokinetic p. d. in millivolts in this and subsequent figures multiply μ/sec. per volt/cm. by 12.6. (In this connection see Northrop and Cullen: J. Gen. Physiol., 1921-22, 4, 638.)

of years on the basis of their wetting properties and cataphoretic behavior. The two types seemed to offer splendid material for the extension of the general theory of the stability of bacterial suspensions.

Accordingly experiments have been performed to test the hypothesis that the stability of suspensions of acid-fast bacteria depends upon conditions more closely resembling those for the stability of dispersions of hydrophobic colloidal particles; and that the varieties with apparently markedly hydrous surfaces form suspensions whose stability depends more definitely upon the hydration factor, than do those of the suspensions of the two types of bacteria studied by Northrop and De Kruif.

Fig. 1. records such an experiment. Washed suspensions in distilled water of two of the hydrophilic bacteria, the dysentery and the colon bacillus, and two acid-fast hydrophobic bacteria, the turtle bacillus and the Arloing strain of avian tubercle bacillus, were mixed with water and with dilute HCl solutions. The concentrations of HCl after mixing, in millimols per liter, are given as abscissae. Agglutination was read after one hour and after 18 hours in the ice box. The electrophoretic mobilities were determined in a microcataphoresis cell[25] following the 18 hours reading. Each suspension was examined in the cataphoresis cell in HCl of the same concentration as that in which the agglutination readings had been made.

It is apparent that only a trace of agglutination of the dysentery bacillus occurred in any acid concentration, although the electrokinetic p. d. was extremely low; in 0.6 millimolar HCl the p. d. for the dysentery bacillus was about 3 millivolts. The colon bacillus showed very little agglutination although the p. d. was minimal; the colon bacillus was stable in distilled water with a p. d. of only about 3 millivolts. It is obvious that the stability of the colon bacillus in distilled water is attributable neither to reduction of the cohesive force by electrolytes nor to a high surface potential charge.

The turtle and avian tubercle bacillus, on the other hand, which in an oil-water interface show marked preferential wetting by the oil,[22], are rapidly aggregated in concentrations of acid sufficient to reduce the p. d. below its critical value. In the case of the avian tubercle bacillus the value of this critical potential seems to be high, nearer that found by Powis[26] for oil drops than that found by Northrop and De Kruif for non-acid fast bacteria.

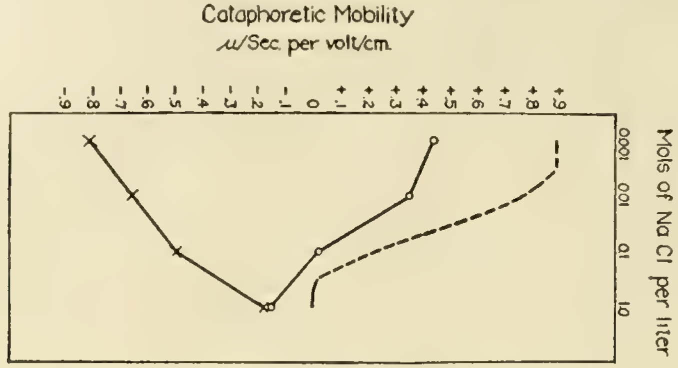

Fig. 2. Effect of salts on p. d. and lack of effect on agglutination of turtle bacillus and avian tubercle bacillus. Bacteria were suspended in 0.01 N HCl to which were added the amounts of NaCl indicated on the axis of abscissae. Circles, turtle bacillus. Crosses, avian tubercle bacillus. The upper broken line is the curve for typhoid bacillus in HCl and NaCl, redrawn from Northrop and De Kruif's [17] Fig. 4, p. 647. Unbroken line, complete agglutation. Broken line, no agglutination. The high electrolyte content inhibited agglutination of the typhoid bacillus, but not that of the hydrophobic acid-fast bacteria.

In Fig. 2 the same two acid-fast bacteria are set up in strongly acid solutions containing graduated concentrations of NaCl. The concentration of HCl after mixing was N/100 in each tube. The NaCl contents in the several tubes were 0.001, 0.01, 0.10 and 1.0 molar, respectively. These experimental conditions were chosen to duplicate as nearly as possible those of Fig. 4 in the paper of Northrop and De Kruif.[17] In our experiment the acid reduced the potential below the critical value for the hydrophobic bacteria and agglutination occurred in all tubes in spite of the very high electrolyte concentration. The corresponding curve in Northrop and De Kruif's Fig. 4 is replotted for contract: with these bacteria no agglutination occurred until the very high "salting out" concentration was reached.

The "rough" variants of the intestinal bacteria differ in their stability relations both from these hydrophobic acid-fast bacteria and from the hydrophilic smooth intestinal forms. The rough variants have electrokinetic p. ds. which are depressed by decreasing pH values and by increasing electrolyte concentrations. See Tables I. and II. In very dilute electrolytes these "rough" bacteria form stable suspensions. They are usually readily aggregated, however, in solutions in which both acid-fast bacteria and the hydrophilic smooth forms are stable.

The general conclusions of this section of the lecture may now be stated. Bacteria exist which display a wide range of surface types, from those which are markedly hydrophobic to those which are markedly hydrophilic. The factors governing the stability of dispersions of the various types in aqueous media are the same as apply to the stability of colloidal particles with similar types of surfaces. The theoretical considerations are those which have been described in the previous section.

Table II.

Precipitability by electrolyte of Bact. typhosum (strain 0 901) in sodium acetate-acetic acid buffer of pH=approx. 5.2.

	0 901 Smooth		0 901 Rough	
Molarity of buffer	Precipitation	Mobility* μ/sec/volt/cm.	Precipitation	Mobility* μ/sec/volt/cm.
0.2	0	0.0	++++	—0.5
0.04	0	—0.1	+++	—1.6
0.02	0	+0.1	++	—2.4
0.01	0	0.0	++	—3.2
0.004	0	+0.1	++	—3.6
0.002	0	+0.5	++	—3.9
0.001	0	—0.1	+	—3.9

Bacteria with strongly hydrophobic surfaces are stabilized in aqueous media chiefly by electrokinetic potential difference. They agglutinate when their electrokinetic potential is reduced below a definite relatively high critical value. Others, of the type studied by Northrop and De

* The sign of the bacterial charge is indicated by — or + before the mobility value.

Precipitation readings made after 20 hours in refrigerator. Data in Tables I and II obtained by Miss Eleanore W. Joffe.

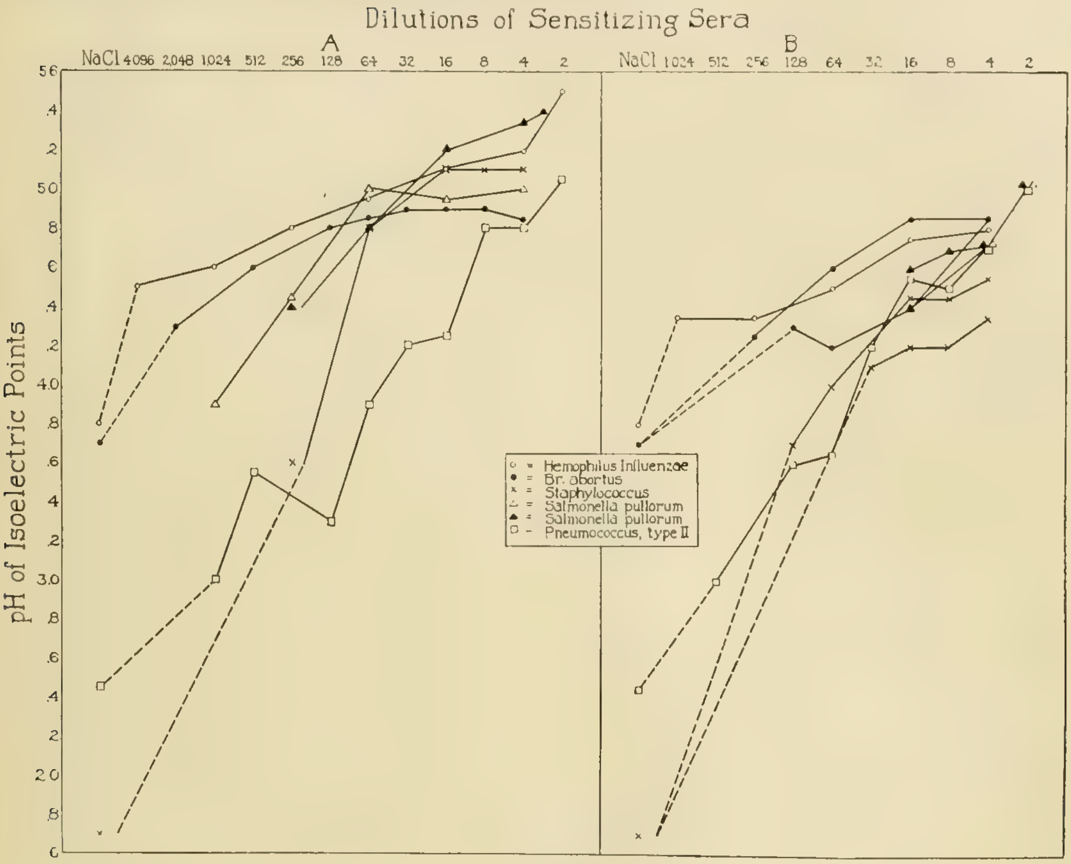

Fig. 3.

Kruif, form suspensions in which both electrokinetic potential difference and hydration are stabilizing factors of primary importance. This is not difficult to understand since evidence has recently been brought forward to indicate that the surfaces of many or most bacteria contain both hydrophobic and hydrophilic components.[27] Finally bacteria exist whose state of surface hydration is the primary stabilizing factor in their dispersions in aqueous media over a wide range of conditions.

The Stability of Suspensions of Sensitized Bacteria

Bacteria within the human or animal body are altered in their surface properties by the defensive mechanisms of the host. The globulins and albumin of the blood are adsorbed to a greater or lesser extent on bacteria with which they come in contact, and the intrinsic surface properties of the bacteria are thus masked by the adsorbed protein. If the infection persists long enough new substances, known as antibodies, which appear to be globulins with physical-chemical differences from normal serum globulins[28], are elaborated. These antibodies possess specific chemical affinities for substances in the bacterial surfaces. The adsorption of the normal blood proteins and the specific chemical combination of antibody-proteins with the bacterial surface are known as serum "sensitization."

Sensitization results in marked changes in the physical properties of the bacterial surfaces, i. e. the sensitized bacteria are more cohesive, their wetting properties are altered, their electrokinetic p. d. is, under the conditions of the usual serological experiment, reduced, and their isoelectric point is shifted to a value near (but often not identical with) that of serum globulin.[28, 29] These changes are consequent upon the formation of a surface deposit of antibody-globulin on the antigen. Since electrokinetic p. d., cohesion (and hydration), are the fundamental factors determining stability, the stability of sensitized would

be expected to differ from that of unsensitized bacteria. As a matter of fact agglutination is the most familiar consequence of combination with antibody.

The remarkable specific chemical affinity between antigen and antibody enables the antibody in exceedingly high dilution to form an effective surface deposit on the antigen. Thus antisera may be prepared which agglutinate typhoid bacilli in a dilution of one volume of serum in a hundred thousand volumes of dilutent. The surface deposit once formed, however, has many points of resemblance to deposits of serum proteins, egg albumin or other proteins formed by non-specific adsorption on bacteria or other particles. The non-specific deposit of serum proteins, in addition to requiring higher concentration of protein to form an equivalent deposit, is in general less firmly held than the specific deposit.

In general with the progressive formation of a surface deposit the electrokinetic p.d. and isoelectric point of the particle approach those of the deposited substance.

The effect of serum sensitization on the isoelectric points of various bacteria are shown in Fig. 3. (cf.Shibley[30]). The bacteria were treated in the left hand side of the figure with serial dilutions of the sera of the patients from which they were isolated: on the right hand side of the figure the results of treatment with normal serum or serum from another disease are shown. A portion of the bacterial suspension was allowed to stand overnight in each serum dilution, the sensitized bacteria were then washed in 0.85% NaCl solution, and their isoelectric points were determined in acetate buffer series with the aid of a Northrop-Kunitz microcataphoresis cell. Before sensitization the staphylococcus retained a negative potential even in N/100 HCl, the pneumococcus was isoelectric between pH 2.0 and 3.0, H. influenzae and Br. abortus between pH 3.0 and 4.0, and S. pullorum had little if any surface p.d. in any buffer used. With progressive sensitization the isoelectric points of all the bacteria converged progressively until values of pH=4.9 to 5.5 were reached after sensitization with the patient's serum, and of pH=4.35 to 5.0 after sensitization with normal serum.

The stability conditions in the case of gelatin adsorbed on collodion particles have been shown by Loeb closely to resemble these of gelatin solutions.[31] Loeb showed on the other hand that collodion particles coated with egg albumin showed the stability relations of denatured albumin rather than those of native albumin. Students of specific bacterial agglutination from Bordet on have been impressed with the fact that sensitized bacteria were aggregated by traces of cations which were alike incapable of precipitating the unsensitized (non-acid fast) bacteria or the serum globulins with which the antibodies are associated. The antibody-globulin combined with antigen has therefore been spoken of by Shibley[30] and others as "denatured."

A given dispersion of particles is stable either if the repelling force (electrokinetic potential difference) is greater than its critical value, or if the cohesive force of the particles ($2\,S.\,\gamma\,AB$) is below its critical value. It follows from this that for aggregation and precipitation to occur both the electrokinetic potential difference must be below its critical value and the work of cohesion must be above its critical value.

A stabilizing or protective film forming substance is one that, under the conditions of test, results in a surface such that either the electrokinetic potential difference is above the critical value for that surface or that the work of cohesion of the surface is below its critical value, or both. A precipitating or sensitizing film forming substance is one such, that under the conditions of test, a surface results which is both below its critical potential and above its critical work of cohesion. It is apparent that one and the same substance may act either as a stabilizing or sensitizing film forming substance depending upon the conditions of test.

Since after combination with bacteria has occurred, the effect of antibody film on stability conditions is entirely analogous to that of other types of films, the above considerations apply to the agglutination of sensitized bacteria. Antibody films which cause agglutination of bacteria do so because they result in surfaces which are both below their critical potentials and above their critical works of cohesion under the conditions of test. Bacteria may also be agglutinated by tannin[32] and it may be shown that in this case also a surface deposit is formed whose electrokinetic p.d. is below and whose cohesion is above its critical value[1].

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Discussion

Dr. Abramson: In discussing the interfacial technique, when you have bacteria partially coated with protein film, haven't you also a protein film at the aqueous-oil interface, and doesn't that enter into the picture? Have you the right to call the interface oil-water in the presence of proteins or other adsorbable substances? A plastic film of some sort should be formed.

Dr. Mudd: Undoubtedly the oil-water interface becomes contaminated with any adsorbable material present. We have had this fact in mind, however, and have taken care to reduce it to a minimum and so to arrange the experiments that it should not vitiate our results.

Dr. Riddle: Do collisions between particles actually occur or do they not from the standpoint of actual visual observation?

Dr. Mudd: I presume you are referring to collisions without aggregation. I do not know of anyone who has written on that point. It would be difficult to tell, of course, because the distances involved would be of the order of 10^{-8} cm. which is considerably beyond the powers even of the ultramicroscope. I doubt if this question could be answered by direct observation.

Dr. Cole: When bacterial agglutination takes place, how rapid is it?

Dr. Mudd: Ordinarily of the order of slow coagulation rather than of rapid coagulation. It is possible, however, to get rapid coagulation of hydrophobic bacteria with acid.

Dr. Cole: This, I should think, would be a case where you would have practically a monodispersed system in which the Smoluchowski theory should follow. Is it followed?

Dr. Abramson: Oliver and Barnard showed that the Smoluchowski theory is followed perfectly in rapid coagulation of red cells.

Dr. Müller: The coagulation of rod-shaped particles has a very much faster rate than for spherical particles. The deviations from Smoluchowski's curve are particularly large at the beginning of the coagulation. Later on the curves approach again the curve for spherical particles.

Dr. Mudd: Bacterial suspensions offer a considerable range of shapes and surface properties favorable for the study of coagulation problems.

Dr. Müller: Would you prefer to assume that a colloid is not a system in thermodynamic equilibrium?

Dr. Mudd: The more usual assumption, which is implicit in the ordinary treatment, is that hydrophobic colloidal systems are metastable. If the particles could come in contact they would aggregate, but the double layer prevents the approach of the micelles. Do you believe it necessary to assume that hydrophobic colloids are in thermodynamic equilibrium?

Dr. Müller: This assumption is not necessary. By giving it up, however, we can not treat a colloid with the help of the ordinary methods of thermodynamics. If a colloid is only in metastable equilibrium, one can consider the problem in the following way: If a colloidal particle approaches another one, it comes first under the influence of repulsive forces, created by the double layers. These repulsive forces can be represented by a

"potential-hill." If the temperature energy of the particle is large enough, the particle can traverse this hill and comes then under the influence of the attractive forces of the surface tension. These forces can be represented by a "potential-valley." This valley is usually so deep, that the particles have not enough kinetic energy to come out of it —which means that coagulation takes place. Hydration decreases the depth of this valley, and it is possible that very strong hydration decreases the depth so much that a particle may again escape from the attraction of the surface forces. That is the case if the temperature motion is larger than the surface forces. Hydration may, therefore, produce stability. If there is no or little hydration, the stability is due to the first potential hill. If this hill is high enough no particle has a large enough kinetic energy to overcome the repulsive forces. My contention is that the ordinary electrostatic forces are not strong enough to present a hill high enough. The stability of large particles is only assured if we take also into account the change of energy of the water due to electrostriction. This effect is similar to hydration; it differs from hydration insofar as it is produced by electrostatic forces, while hydration is due to molecular forces.

My proposed theory is qualitatively in agreement with Dr. Mudd's point of view. The diference is only in the quantitative relationship. The consideration of the electrostriction is necessary to explain a sufficiently high potential-barrier. The height of the hill is again determined by the ζ-potential. The dependence of the stability on the properties of the double layer is, however, more complicated than if only electrostatic forces are considered. It seems probable that not only the ζ-potential, but also the thickness of the double layer is equally important for the determination of stability. Kruyt has, for instance, found colloids which coagulated at a high ζ-potential, and were stable at a low ζ-potential.

Dr. Mudd: By treating our hydrophilic bacteria with progressive concentrations of specific immune serum under the proper conditions, it is possible to get agglutination in parallel with either an increasing negative or positive ζ-potential. In this case, however, we are progressively forming a new surface of hydrophobic protein upon the original hydrophilic bacterial surface.

Dr. Cohen: What happens to the surface energy when particles aggregate?

Dr. Müller: I believe the energy is so small that you can not say very closely.

Dr. Cohen: Since numerous particles are involved the aggregate effect may be appreciable.

Dr. Müller: One would expect that there would be a heating effect.

Dr. Cohen: Dr .Mudd speaks of certain bacteria as highly hydrated (hydrophilic) and certain others as hydrophobic. The contrast between this view and Dr. Abramson's discussion of proteins in water, which leaves water out of the picture, is puzzling. Why was water ignored?

Dr. Abramson: It might affect the surface as far as agglutination is concerned, but it apparently does not change the time average of the net charge. I can't visualize the exact hydration mechanism of proteins. Certainly very little is actually known of the hydration of proteins. There is one interesting point I would like to follow up. This is Northrup's curve for mobilities. The charge is not given by this curve. This curve gives the ζ-potential, and note that you get coagulation when the potential drops, say, from a relative value of 0.8 μ per second to a value of 0.3 μ per second. If you change the potential one-half you get coagulation. In the literature, you frequently see statements to the effect that the bacteria are discharged. This is not correct, because the charge is proportional to

$$\sqrt{c}\,\sinh\frac{\zeta e}{k\,T}$$

Actually where you get agglutination the charge is at a maximum or near its limit in value, whereas it is the potential which is depressed.

Dr. Müller: Nevertheless, the ζ-potential is the important thing for agglutination, not the charge.

Dr. Abramson: But the charge is not decreased as is frequently stated in the literature, nor need the ζ-potential be decreased; it can also increase as in the case of sensitized bacteria.

Dr. Blinks: Do these experiments work as well with dead bacteria?

Dr. Mudd: Yes, we use either living or dead bacteria.

Dr. Briggs: In the case of the smooth strains of bacteria your observations show that they exhibit zero electrokinetic potential throughout a wide range of pH change. This is certainly an unusual surface. Just what sort of a surface do you picture as existing there that could show such properties?

Dr. Mudd: I was in hope some of you would help us solve this perplexing question. However, many bacteria are known to contain polysaccharids in their surfaces, and it seems conceivable that these may neither themselves be ionogenic nor have a preferential adsorption affinity for ions.

Dr. Briggs: But polysaccharids do show ζ-potentials and these change radically with pH.

How do smooth and rough strains act at the interface between oil and water?

Dr. Mudd: The smooth hydrophilic strains are preferentially wet by the water; they do not pass into the oil. A comparison of the corresponding rough strains in this respect would be very desirable but has not yet been made.

Dr. Cohen: With reference to capsulated bacteria of the smooth type, is it not true that the capsules are largely composed of polysaccharids which are highly hydrated?

Dr. Mudd: I think this is true of the pneumococci and Friedlander's bacilli at least. Their capsular material is quite soluble.

Dr. Cohen: What I mean is that one can often see on capsulated bacteria a capsule which is many diameters thicker than the cell itself. Recent evidence shows that this capsular material consists largely of polysaccharids. Under the microscope it often appears highly refractive, and there is much evidence that it is loaded with moisture. Having in mind also the chemical constitution and configuration of these carbohydrates, one may picture that the capsule is in essence a radially (not concentrically) stratified conglomerate of elongated polysaccharids micelles projecting into the culture medium. Into this sort of radial structure water could penetrate some distance; in addition, the polysaccharid itself is highly hydrous, therefore the actual boundary between this type of capsule and the surrounding liquid medium would be rather hard to define.

Dr. Briggs: For the surface to exhibit no ζ-potential as a result of surface hydration would require that the double layer must be completely within the layer of adsorbed water. Is that possible?

Dr. Müller: It seems possible that if we have very strong hydration, the double layer is entirely inside the rigidity boundary. Since we must have a small dielectric constant in the adsorbed layer, this double layer is practically a rigid one.

Dr. Blinks: What happens in the migration of a jell of agar?

Dr. Abramson: It has a pretty high mobility.

Dr. Briggs: If such a strongly adsorbed layer of water does exist at the surface of the particle, it should in reality form a phase, the properties of which would be different from that of the bulk of the water. Selective adsorption of the ions in solution should occur across this new boundary, and give rise to a ζ-potential.

Dr. Abramson: Ice shows electrophoresis in water.

Dr. Müller: In ice the water molecules are not rigidly bound as in hydration. For low frequencies ice has the same large dielectric constant as water, which indicates that the H_2O molecules in ice can rotate.

END OF COLD SPRING HARBOR SECTION

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Jean M. Clark, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

Printed by the Darwin Press, New Bedford

THE COLLECTING NET SCHOLARSHIPS

The scholarships which we initiated in 1927 seem to have become a "permanent institution" although we never know for certain at the beginning of the season that we can accumulate five or six hundred dollars for award in September.

Owing to our relations with the Biological Laboratory at Cold Spring Harbor it has been decided to have one scholarship of $100.00 awarded to a student working at that institution. The award will be made by Dr. Harris and a committee appointed by him. Further, it has been decided that the scholarship winners from both institutions may have the privilege of working at either Woods Hole or Cold Spring Harbor, providing their application for work is accepted by the chosen laboratory. We look forward to the time when both institutions will grant scholarship holders a free research table, so that they can use the whole hundred dollars to meet their travelling and living expenses.

We owe a debt of gratitude to Vera Warbasse, Alfred Compton and Thomas Ratcliffe who form the executive committee of the Penzance Players, and their staff, who presented George Bernard Shaw's play "You Never Can Tell" in the interests of our scholarship fund. We hope to be able to announce next week the sum of money which will be realized from this source, but we are confident that it will be enough to take care of one or two scholarships.

Contributions—small or large, tiny or huge—will be greatly appreciated. It is our wish to accumulate a little more money each year than is needed for award at the end of the summer, in order to build up gradually a nucleus for our endowment fund.

Introducing

Dr. Joseph Spek who is professor of zoology at the Zoological Institute, University of Heidelberg, and one of the editors of *Protoplasma*. Arriving in this country in the Fall of 1932, he worked at the laboratory of Dr. Robert Chambers at New York University during the Winter, injecting amoeba to determine the differences in pH of constituents of protoplasm. Since his arrival in Woods Hole on May 30 he has been carrying out vital staining experiments on the eggs of various organisms: Asterias, Nereis, Loligo, etc.

Dr. Spek lectures on general zoology at the University of Heidelberg, and is interested primarily in the fields of cytology and experimental embryology. He is the author of the articles on experimental embryology in Gellhorn's textbook: "Lehrbuch der allgemeinen Physiologie." *Protoplasma,* which is published in Berlin, is edited by Drs. Spek and Weber of the University of Graz with the collaboration of Drs. Chambers and Seifriz in this country.

Dr. Spek will deliver his lecture in German on Friday concerning protoplasmic differentiation in egg cells during early development. He was asked especially to speak in German, since it was thought that Laboratory members, beside being interested in the subject itself, would also appreciate the opportunity of hearing scientific German.

Dr. Spek plans to sail for Germany from New York on the *S. S. General Steuben* on August 28.

R. G.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the hole turns to run from Buzzards Bay to Vineyard Sound:

	A. M.	P. M.
August 5	4:20	4:31
August 6	5:12	5:25
August 7	5:59	6:15
August 8	6:46	7:05
August 9	7:32	7:55
August 10	8:17	8:42
August 11	9:04	9:35
August 12	9:51	10:28
August 13	10:43	11:25
August 14	11:36	....

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

PENZANCE PLAYERS PRESENT PLAY FOR SCHOLARSHIP FUND

The Penzance Players presented Shaw's brilliant comedy, "You Never Can Tell" at the Marine Biological Laboratory last Monday for the benefit of The Collecting Net Scholarship Fund. It was a gracious gesture on the part of these young people, and it was plain to observe that the large and friendly audience of summer residents, laboratory workers, and townspeople appreciated not only the high calibre of the production itself but also the co-operative and social spirit behind it.

The play was ably directed by Mrs. George A. Baitsell, and the cast consisted of Vera Warbasse as Gloria Clandon, Thomas G. Ratcliffe as Mr. Valentine, Alfred Compton as the waiter, Peggy Clark as Mrs. Clandon, Faith Adams and Manton Copeland, Jr., as the twins Dolly and Phil, Eric Warbasse as the father, Mr. Crampton, Robert Giddings as Mr. McComas, Frederick Copeland as Mr. Bohun, Alice Cooper as the parlormaid, and William Chambers as the second waiter. The production was staged by Alfred Compton, with Robert Chambers as stage manager, Maynard Riggs in charge of properties, and J. Warren Sever in charge of lighting. Settings were constructed by J. Wister Meigs with the aid of Alice Gigger and J. Warren Sever. Charlotte Fitch was in charge of make-up. The business manager was Peggy Clark, who was assisted in her work by Alice Gigger and Sebree Robbins. The executive committee of the Penzance Players group consists of three of the actors, Vera Warbasse, Thomas Ratcliffe, and Alfred Compton.

Selections by the Hawthorne String Quartette were heard between the acts. The action was of course laid in England and the set for the terrace of the Marine Hotel in Act II was especially pleasing to the eye. The plot concerns the unconventional escapades of the offspring of a determined feminist, Mrs. Clandon. Gloria, the eldest, is at heart old-fashioned, and Shaw permits the love affair of Mr. Valentine and this daughter to provide a happy ending for the play, but not before he has delivered himself of some typically witty remarks on feminism, lawyers, love, etc. The Penzance Players entered into the spirit of the thing heartily, and a splendid time was had by them and by their delighted audience.

Dr. Elmer J. Lund, professor of physiology at the University of Texas, is visiting for a few days in Woods Hole. Dr. Lund is at present investigating the origin and function of bioelectric current.

ITEMS OF INTEREST

When the Third International Congress for Experimental Cytology meets in Cambridge, England, from August 21 to 26, several investigators who have been working at Woods Hole during the earlier part of the summer will be present in an official capacity. Dr. Robert Chambers will read a paper on "Some features of cell permeability in relation to kidney function," Dr. L. Michaelis, a paper on "The reduction intensity of the living cell," Dr. S. C. Brooks, one on "The relation between ions and potential differences across plasma membranes," and Dr. C. C. Speidel will show his moving pictures of nerve growth and repair.

Dr. Clarence E. McClung, professor of zoology and director of the zoological laboratory at the University of Pennsylvania, left Philadelphia with Mrs. McClung on June 19 for Tokyo, Japan. He will spenr the next year there as professor of zoology at Keio University, under a grant from the Rockefeller Foundation.

Although Tokyo is their ultimate destination, the McClungs will not arrive there until the beginning of the fall term, in September. On their way they visited relatives in Kansas and Washington, and are now spending the rest of the summer vacation in Hawaii. Miss Irene Corey, Dr. McClung's secretary, joined them when they sailed from Seattle in the middle of July, and will also be in Tokyo this year.

The grant under which Dr. McClung is working has provided four previous professorships at Keio University. The other zoologists who have held the position are Dr. Pearce, of Duke University, Dr. Tennent, of Bryn Mawr College, Dr. Jennings, of the Johns Hopkins University, and Dr. Curtis, of the University of Missouri.

Dr. D. H. Wenrich, professor of zoology at the University of Pennsylvania, is spending the summer with his family in Kansas. During his visit he has been working at the University of Kansas, collecting and studying parasitic protozoa.

Dr. Arthur H. Compton, professor of physics at the University of Chicago, recently visited Woods Hole. While he was here he was concerned with the fate of the attempted balloon ascension in Chicago since it contained some of his cosmic ray recording apparatus. Dr. Compton has been teaching in the summer school at Columbia University, and his trip to Woods Hole was motivated by an interest in the group of scientific workers, as well as by a desire to visit his friends here.

Supplementary Directory for the Marine Biological Laboratory

INVESTIGATORS

Albaum, H. G. fel. biol. Brooklyn. OM 41. Dr 1.
Bacq, Z. M. Nat. Res. Fel. Liege Br 114. D 317.
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Blinks, L. R. assoc. gen. phys. Rockefeller Inst. Br 209. D 203.
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Hall, E. K. instr. anat. Louisville Med. OM 31. D 209.
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Brooks, Virginia C. Wilson (Pennsylvania) H 4.
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Clark, Frances J. Rochester. H 9.
Collings, W. D. asst. zool. DePauw. Hilton, Millfield.
Cowles, Janet M. Hopkins. D 215.
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Di Paola, Rose M. Hunter. K 10.
Field, Mary F. Grinnell, Bar Neck.
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Giddings, W. P. Amherst. (Quisset).
Henderson, A. R. asst. zool. Yale. Ka 22.
Horton, R. G. Williams. A 106.
Hunter, F. R. grad. genetics Calif. Inst. Tech. D 107.
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Todd, R. E. Moses Brown School. Wilde.
Trezise, W. J. asst. zool. Hopkins. Robinson, Quisset.
Van Deventer, W. C. grad. zool. Illinois. Backus, Glendon.
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Whittinghill, M. instr. zool. Dartmouth. Clough, Millfield.
Williams, Inez W. grad. entomology. Mass State.
Williams, Marguerite. grad. zool. Iowa. W B.
Wing, L. T. Harvard. Gifford, Millfield.
Woodside, G. L. teach. fel. zool. Harvard. K 7.

THE COLLECTING NET

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ORGANIZERS AND INHERENT POTENCIES IN THE EMBRYONIC DEVELOPMENT OF AMPHIBIANS

DR. OSCAR SCHOTTÉ
Sterling Fellow in Zoology, Yale University

Animal embryology has for its aim the description of all the changes and transformations which the egg undergoes until it reaches the definite form and organization of its species. One division of this science, the morphology of development, has the task of describing how from the initial system of a given complexity of structure, the egg, a new organism arises, provided with regions and organs fundamentally different from the primitive structure of the egg. This descriptive embryology has reached an amazing stage of perfection.

The human mind, however, cannot be satisfied by the mere description of a phenomena. The facts of development are too striking; their astonishing diversity brings up too many questions not to demand an explanation of *how* development occurs, and what are the reasons lying behind this amazing succession

M. B. L. Calendar

TUESDAY, AUG. 15, 8:00 P. M.

Seminar: Dr. A. H. Palmer: "The isolation of a crystalline globulin from the albumin fraction of cow's milk."

Dr. Paul Reznikoff: "Studies in iron metabolism in humans."

Dr. Marie Krogh: "The hormonal connection between the pituitary and the thyroid."

Dr. F. E. Chidester: "Anterior pituitary like hormone effects."

FRIDAY, AUG. 18, 8:00 P. M.

Lecture: Dr. John H. Northrop: "Evidence of the protein nature of pepsin and trypsin."

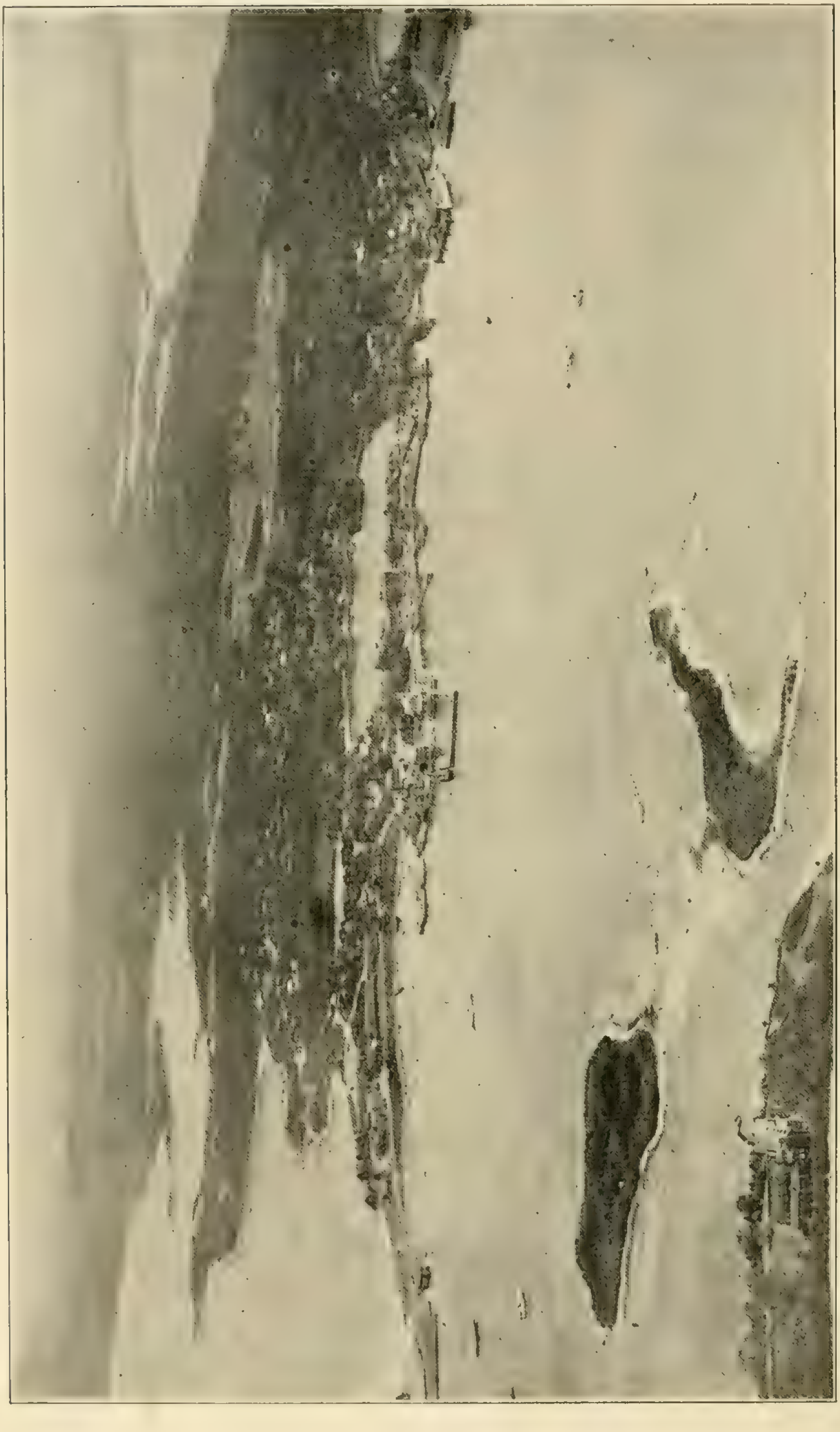
THE EFFECT OF FAT SOLVENTS UPON THE FIXATION OF MITOCHONDRIA

DR. CONWAY ZIRKLE
Associate Professor of Botany, University of Pennsylvania

There are many uncontrolled variables in the usual cytological fixation. These rarely affect the preservation of chromatin, however, as the methods in general use have been derived empirically and have been carefully selected for the reliability with which they preserve the nuclear components. On the other hand, the cytoplasm and its inclusions have been relatively neglected, and it is still customary to represent the cytoplasm by a fixation image where its essential structure is destroyed and the inclusions, particularly the mitochondria, dissolved. A number of biological problems await dependable methods of fixing mitochondria, for at present the absence of mitochondria in a cytological preparation is no evidence that they were not in the original specimen.

The presence in the fixing fluid of such fat solvents as the aliphatic (*Continued on Page 202*)

TABLE OF CONTENTS



AN AERIAL VIEW OF WOODS HOLE
showing a bit of Nonamesset in the foreground. This photograph was taken in 1929 for THE COLLECTING NET by a photographer of the United States Army Air Corps.

of transformations which are so characteristic for all embryonic happenings. From the desire to understand the mechanism of this phenomena and from the clash of opinions in the interpretation of facts of development, is born a new division of embryology, the physiology of development. Some problems dealing with the physiology of development of the Amphibian egg, exclusively, will be the object of our study this evening.

If one uses the methods of vital staining to study the fate of different parts of a living Amphibian egg, one can observe during the later development how a stained spot moves, changes its form and travels often quite far from its initial place to arrive eventually at the region in which it will stay to achieve its final evolution. By studying these movements systematically one can succeed in establishing a kind of a geographical map of the different parts of the egg which will build up the future animal. It was the merit of W. Vogt to establish thus the complete pattern of organ rudiments for both the Urodele and the Anuran eggs. This "map" is perfectly reliable and allows us to make transplantations on definite parts of the young embryo, long before we can recognize on it the slightest trace of its future form or its future organs. The fact that we know perfectly that certain parts are going to become brain or eye or mouth region indicates that there is a mosaic in the egg, only definite groups of cells being destined to become these organs.

Another experiment of W. Vogt seems to confirm this mosaic tendency in the Amphibian egg. In this experiment Vogt places the egg in a dish in which, by a partition, a notable difference of temperature is realised. The egg is placed directly under the partition so that one half of it is exposed to a temperature of 8° C and the other half to 15°. He could observe that the half of the egg exposed to a higher temperature had already developed a spinal cord and the rudiment of a brain, while the half exposed to colder water was still in the blastula stage. This outcome of the experiment indicated that the parts of the egg can realise their common aims in perfect independence of each other. Since the different regions of one half of the egg are here shown to be able to reach their definite stage without the interaction of the other half, this independence in development indicates a pronounced tendency to realise the pattern of the egg in a "mosaic" manner.

The method of local injuries is another means of investigation, apparently confirming the mosaic interpretation of development for the Amphibian egg. Indeed, Brachet in a large series of experiments performed on Anuran eggs, and Suzuki on Urodele eggs, could show that each time a part of the embyro was destroyed, (besides some exceptions which could be explained) the larva of the injured egg will show later a deficiency of structure corresponding to the area of presumptive organ primordiums which was destroyed.

But the observation of this "embryonic segregation" (F. R. Lillie) by both Vogt's methods or by following the fate of the embryo through the methods of local injuries, gives us only a "suggestive criterion," as Professor F. Lillie expresses it, of the happenings during the development. What lies behind this segregation remains hidden from us.

A second series of investigations representing a further attempt to give us some other information about facts of development, consists in submitting parts of the egg to the method of interplantation or to the method of tissue culture. In the first place, pieces of presumptive ectoderm, entoderm or mesoderm are removed from the donor embryo and explanted into ectodermal vesicles (Bautzmann), into the orbital cavity of an elder larva (Dürken, Kusche) or into the general cavity of larvae (Holtfreter). In all these cases, the presumptive fate of the explanted organ primordiums is changed in the most bewildering manner: ectodermal formations giving birth even to chorda differentiations, entoderm to mesoderm, etc.

It is not necessary to insist upon these formations which Bautzmann called "bedeutungsfremde Selbstdifferenzierungen". They are not "Selbstdifferenzierungen"—independent differentiations —because they are under a constant action of uncontrollable factors, and so they lose their significance. The conditions of these experiments do not answer at all to the definitions of a "radical criterion," such as F. R. Lillie gave it.

These latter conditions are fulfilled in a second series of explanations performed also by Holtfreter, in which he cultivated parts of the blastula or gastrula with known presumptive fate, in vitro, in a salt solution. There he obtained very different results:—ectoderm produces in vitro only ectodermal formations, presumptive medullary plate ectodermal formations and nervous tissue. Presumptive mesoderm in tissue culture will form chorda, connective tissue and muscles, but will never produce nervous system.

We see now that from the one side we distinguish in the Amphibian egg a pre-established pattern of organ primordiums, that this pattern cannot be destroyed without giving birth to deficient larvae, but on the other side we see already that the elements of this pattern of organ primordiums, if submitted to different conditions outside of the egg, manifest potencies which are dif-

ferent from what their presumptive fate indicates.

Up to this point, I have avoided bringing up any question of causality in this embryonic development. Yet, the physiologically minded embryologist must ask: What is the nature of factors which in some cases make the egg develop like the unrolling of a pre-established and regulated-in-advance mechanism, while in other instances the same parts of the egg develop into very different organs from those they are supposed to, and in some further cases, they may go absolutely wild?

Here a masterful piece of research, inaugurated by Spemann, and continued in constant collaboration with him by his followers, seems to have given, at least for the Amphibian egg, a very clear answer.

Spemann invented in many years of patient effort a remarkably simple method of operating on eggs of the European newt, *Triton*. By using in the beginning eggs of the same species, *Triton taeniatus*, but with marked shades of coloration, so as to make sure of the eventual fate of the transplanted piece, Spemann explored the surface of the young gastrula in many transplantation experiments. By transplanting, for instance, from one gastrula, presumptive abdominal skin in the region of the future medullary plate of another gastrula, the transformation of this presumptive epidermis into spinal cord, or brain, or an eye, has been observed by Spemann. On the other hand, presumptive brain could be transformed into simple belly skin, if transplanted into the abdominal region.

There is one region, however, on the gastrula which behaves very differently from that described above—this is the region of the upper or dorsal lip of the blastopore. When Spemann grafted a piece of this region, he observed that it did not become assimilated to the new surroundings, but that it behaved exactly as did the dorsal lip in the normal development; that is, it became tucked in beneath the ectoderm, underlying the latter, and induced the ventral ectoderm and the mesoderm to form a new medullary plate together with the axial organs of a new embryo. The dorsal lip of the blastopore organizes by its direct action a secondary embryo, for which reason it is called an "organizer."

The action of the organizer seems to proceed with an elementary force—not only do we see a medullary plate arising at the expense of an abdominal skin, but eyes, ears, noses, a whole head with mouth and gills are formed; and if the embryo can be kept long enough, a secondary gut, even a functional heart will be observed. More than that, it is not necessary that one implant to a *Triton* gastrula, the organizer of a *Triton;* one can use for the same purpose the organizer of a frog or of a toad, or vice-versa.

No wonder then that Spemann when he first saw these inductions thought that he had discovered the organizing principle of development itself. Indeed, when we consider that in recent papers, Fankhauser working on the Urodele egg and Pasteels, on the Anuran egg have been able to disclose the localization of the organizer in the unsegmented egg as early as a few minutes after fertilization, and, further, that these authors could actually prove that even in such early stages the presence of the organizer is an unavoidable condition for the development of the totality of organs, then we will understand why the word "organizer" was for us wrapped up in a veil of mystery, which only recently has been dispelled by a new and sensational discovery, coming again from Freiburg.

If I may recapitulate the previous results, we see that the various regions of the Amphibian gastrula manifest, when left alone to perform their normal development, a tendency to form from the given mass of cells always the same organ (Vogt); that these same regions, when detached from the embryo, develop:

(a) according to the new environment if they are transplanted to another embryo of the same species (Spemann);

(b) if submitted to a vigorous control of tissue culture (Holtfreter) they manifest larger but yet restricted tendencies;

(c) they will show up wild tendencies of differentiation in every possible direction if transplanted into some cavities of older larvae (Dürken, Kusche, Bautzmann and Holtfreter);

and lastly, (d) that these same parts of the embryo, when submitted to the vigorous action of the organizer are apparently compelled to develop into harmonious formations representing the essential features of a complete embryo (Spemann).

Thus, the question arises, what are the factors which fix and limit the possibilities of segregation in the normal development and in some experimental conditions; and also, what is the nature and the mode of action of these factors producing such an amazing reversal of potencies, as shown in Spemann's experiments of induction.

In other words, we shall have a perfect understanding of the mechanism of development when we know the role of the inherent potencies of the egg, together with the nature and the role of the organizers.

It was not my intention to speak to you about the intimate nature of the organizers, but two preliminary notes coming from Freiburg which

reached us only some days ago demand special consideration.

In several laboratories, an intensive study was carried on to establish the intimate nature of the organizer. Indeed, Spemann could obtain inductions with frozen and dried organizers; furthermore Holtfreter could show that even non-organizing parts of the embryo can acquire this property after being killed by heat, for instance. A piece of presumptive ectoderm overlapping a dead piece, even of entoderm, showed obvious inductions into a medullary plate. These and some other facts made it likely that a substance of chemical nature was involved in the action of the organizer. The association with a skilled chemist of Freiburg brought the desired results and after a vast series of experiments, it was announced last month that the acting agent which produces inductions is glycogen. Indeed, glycogen dissolved into gelatine and introduced into the blastocoel of a gastrula produces the same medullary plate as does the upper blastoporal lip. Fischer and Wehmeier attribute this action of the glycogen to a glycolyse taking place on the points of contact of the overlapping ectoderm with this substance.

If thus, at least a first, decisive step is made toward the knowledge of the intimate nature of the organizer, a series of problems dealing with the manner in which this "organizing substance" acts still remains open.

In a recent paper, Spemann could show that a part of the upper blastoporal lip which normally would become the underlying mes-entoderm of the head, so-called head organizer, can induce in the region of the trunk brain with eyes and ears. On the other hand, however, trunk organizer brought into the region of the head induces there also a head formation. Thus it becomes plain by these experiments that if the organizer can manifest some specificity in its action, an undoubtful counteraction of the host cannot be denied.

If head organizer can induce a head in the trunk region, whereas the trunk organizer induces in the trunk region only a spinal cord, there seems to be an indication that the organizer can actually direct the fate of the regions upon which it acts. Yet, in all these experiments, a clear answer about the action of organizers is always disturbed by the manifestation of factors due to the action of the inherent potencies of the host.

In 1921 Spemann attempted an experiment which was intended to solve this question. In this heteroplastic experiment, he changed tissues between two species of *Triton*, the incriminated species being *T. taeniatus* acting as host and *T. cristatus* acting as donor. The result of transplantation of presumptive abdominal skin of *cristatus* into the brain region of *taeniatus* shows that while the belly skin of the donor is actually transformed into brain in the foreign host, it keeps its own specific characteristics. Belly skin of *cristatus* is transformed into brain, yet not into *taeniatus* brain, but into a typical *cristatus* brain. This shows that the organizing action of the host on the transplant is of a restricted nature, insofar as this action consists merely in transforming the foreign transplant into an organ which is fixed by its surroundings. But how this organ is constructed, its size, general shape and the disposition of its cells, is a task left to the donor's tissues. While of extreme importance, this experiment could not give a very definite idea about the factors acting in the phenomena of induction. Both host and donor possessing in this case the same organ—brain for instance—they must possess also the same organizers to realize these common organs. Thus, only the different morphological tendencies of host and donor organs were revealed in the heteroplastic transplantation experiment. In order to discriminate between the action, in the process of induction, of the organizer versus inherent potencies, it was necessary to find an experiment in which transplantations could be performed between embryos which do not possess common organs or in which these common organs are fundamentally different. These conditions are realized in transplantation, in the head region, between Anuran and Urodele embryos.

Indeed, there are three organs of Anuran embryos which are not common to the Urodele larvae and which are all situated in the upper and lower head region. These are suckers, the operculum and the mouth armament of a type not found in the Urodele mouth.

As early as 1921, Spemann had foreseen some of the possibilities of the xenoplastic experiment; at that time, however, the preliminary conditions for carrying out this investigation were not yet realized—there were some experimental and some theoretical difficulties which had to be overcome first. Since I was fortunate enough to work out a method of transplantation on young stages of Anuran eggs, and since I could show that the same conditions of only labile determination as previously shown on Urodele embryos were also realized in this type of egg, Professor Spemann suggested that I perform transplantations between frogs and salamanders. In these operations the donors were always in the gastrula stage, while the host varied from the stage of young gastrula to late neurula.

In a first series of experiments I performed xenoplastic inductions by transplanting the upper blastoporal lip from either Urodele or Anuran donor into the opposite host. Secondary embryos with a complete set of organs, as in a homeo-

plastic experiment, could be obtained. As an interesting feature, the induction of suckers in the ventral region of a toad by a Urodele organizer could be observed. Furthermore, in both Anuran and Urodele embryos, presumptive abdominal ectoderm from gastrulae was observed to be transformed, if transplanted into the given region, into structures of corresponding nature, as xenoplastically induced lenses, ear vesicles, nasal placodes and pituitary glands.

It was necessary to perform these experiments in order to show that tissues of Urodele and Anuran embryos can develop together well enough to succeed in forming even such a compound organ as an eye. Only now were the preliminary conditions for the second and most important part of this research realized.

In the lower ventral region of the head of young Anuran embryos, ectodermic cells become partly glandular and secrete a mucus by means of which larvae adhere to various objects. The same transformation into a sucker is observed, always in the same region of the lower face, if one transplants presumptive abdominal skin of an Anuran gastrula into this region of a *Triton* or *Amblystoma* embryo, where evidently no such suckers can be found. These induced formations are not only histologically, morphologically and functionally typical suckers of Anuran tadpoles, but they reveal always the specific, often very striking features characteristic of the used donor. Indeed, the sucker induced in the Urodele at the expense of presumptive abdominal epidermis of *Rana esculenta* will be very different from the sucker obtained on the same host from *Bombinator pachypus*. The same observations have been made for the American species, a sucker induced on *Amblystoma* from *Hyla crucifer* tissues being very different from a sucker obtained from presumptive abdominal ectoderm of *Rana sylvatica*. These induced suckers function perfectly, since the secretion of an abundant mucus has been observed in many instances.

If the transplant from the same region of the frog egg happens to cover the upper side of the head of the Urodele, one sees about 6-7days later the formation of a cutaneous pocket at the expense of the transplanted tissue. As the form and position of this pocket is always the same and corresponds exactly to the position of an Anuran operculum, this formation is to be diagnosed as an induced Anuran operculum in a region of the Urodele head in which no such thing is expected and where it has no function. The histological study of such cases shows that this formation is situated in a region corresponding to the exact place in which the internal gills of the tadpole were to be covered.

If now the presumptive abdominal skin is made to cover the mouth region of *Amblystoma*, very striking transformations of the ectoderm will be observed. In a first case, the upper and lower jaw from the right side of the mouth were covered by the transplant, and we observed consecutively the formation of two horny jaws and lips covered with numerous horny teeth. In another case, I observed the formation of a real Anuran beak showing the characteristic conical proboscis with rows bearing horny teeth ,and on the free edge of the lips, especially in the corner, typical Anuran fleshy papillae, probably tactile in function, all in striking contrast to the *Amblystoma* mouth.

In another case only the upper jaw was covered with Anuran tissues and yet two horny jaws were found on the upper mandible of the host. Transverse sections through this animal showed on the lower jaw the formation of Urodele teeth, while on the upper jaw we noticed horny teeth, formed each time by the modification of a single epithelial cell in the shape of a hollow cone. It is, as in a typical Anuran jaw, a strong curved band of cornified epithelium. The cutting edge of such a jaw is formed by a row of horny teeth, very similar to those of the lips, but placed so closely side by side as to form a continuous blade.

All the necessary precautions having been taken to avoid the transplantation of presumptive sucker, operculum or mouth region of Anura to Urodele, the demonstration I have given you shows with sufficient evidence that any Urodele host, in the head region of which one transplants presumptive abdominal skin of any Anuran gastrula, is capable of inducing there three organs which are typical for Anuran larvae, but which are lacking in the Urodele. How shall we explain these somewhat astonishing facts?

A priori, it was not absolutely impossible to admit the presence, in the Urodele organism, of a specific organizer for these foreign organs. But if one considers the matter more closely, this presumption becomes highly improbable. If one could admit, for instance, that the balancer region could at the same time be bearer of the organizing factors for suckers, then how shall we explain the case in which two balancers were observed together with two suckers, as shown in one of my experiments?

Furthermore, if the organizers for balancers and for suckers were common, then the transplantation of undetermined tissue of Anuran gastrulae on the balancer, and of gastrula ectoderm of Urodele on suckers, must have been followed by the induction of the homologous organ. This is not what occurs if these conditions are realized. Indeed, in one case of transplantation of *Triton* gastrula ectoderm on a *Bombinator*, it was seen that half of the left sucker was covered by Uro-

dele tissue. And yet, the transverse section through this region shows no sign of differentiation of the Urodele tissue into a balancer. The inverse condition is realized in the case of an Urodele having half of the balancer covered by Anuran tissue; here also the presumptive abdominal skin of the gastrula of the donor did not differentiate in anything else as simple skin and and not in suckers.

It would be very difficult also to admit the possibility of an operculum organizer in the upper head region of Amblystoma, while the gills are produced far away from this region and are never covered by it.

And how can we admit that the Urodele, having already realized the mouth armament of his own, has still some organizers in reserve to form on the edge of its jaw some supplementary horny teeth and jaws, as is visible on one of the demonstrated cases?

Thus, we must exclude the possibility of organizers for these foreign organs.

The explanation of these strange inductions is already given by the analogy with Spemann's case in 1921: just as we saw that the tissues of *T. cristatus* are unable to form in the *taeniatus* brain region a *taeniatus* brain, but remain characteristic for a brain of the donor's species, so we can admit by analogy the assimilation of a piece of abdominal ectoderm into a face region in the Urodele. But, it remains Anuran material, and therefore the tissues have to develop organs belonging to the same region of an Anuran larva—these are mouth armament and suckers for the lower and middle face region and operculums for the upper and lateral head region. Spemann names this kind of induction a "komplexer Situationsreiz." That is very easy to say in German, but difficult to translate into English, though we could try to say "a complex stimulus of localization."

While this explanation seems to be rather verbal, it gives a satisfactory account of all the facts observed until now, and of some new experiments I performed this year and of which some aspects are still in course of investigation.

I was very fortunate to find in this country two kinds of eggs which present such exceptional differences of size, that some very interesting problems of development could be carried out on account of this size difference. Indeed, the eggs of the common peeper, or tree-frog, *Hyla crucifer*, are quite exactly three times as small in diameter as the eggs of the common salamander, *Amblystoma punctatum.* In these experiments, which I will report very briefly, I used *Amblystoma* as host, and young gastrulae of *Hyla* as donor. To perform this operation, that is, in order to cover with the transplant approximately the face region of *Amblystoma,* it was necessary to remove nearly the whole presumptive ectoderm, including the presumptive medullary plate. The result of this operation is surprising; one observes the formation of suckers which are typical *Hyla* suckers in their morphological characteristics and in their general shape, but about three times larger than their normal size on *Hyla!* The number of cells at the expense of which these suckers are formed is also about three times greater than is normally observed on *Hyla* embryos.

Similar results were obtained also with other organs, like lenses, nasal placodes, ear vesicles and mouth organs.

At first sight, this observation is contradictory to Harrison's classical experiments on limb transplantation between *A. punctatum* and *A. tigrinum.* You recall that if Harrison transplants the limb bud from the big *tigrinum* on the small *punctatum* he obtains the formation of a huge limb on the small host. The reciprocal experiment gives corresponding results. There is a fundamental difference, however, between these two series of results: Harrison's results are due to a phenomena of differential growth, while in my experiment, we observe a phenomena of induction of a piece of presumptive abdominal ectoderm into a face region on a large animal. Whether this donor comes from a large or from a small animal does not matter. What is important is that I had to remove nearly the whole body ectoderm in order to cover approximately the huge area of the *Amblystoma* face region. Under the organizing action of the host this large piece is transformed into a perfectly harmoniously adapted face region on the new host. On this face region it develops new organs, suckers and mouth armament, characteristic of the donor, but corresponding to the size of the host and not to the size of the donor.

I have said that the aim of this series of experiments was to distinguish between the part of the inherent potencies and of the organizers in the Amphibian development. Was this expectation justified? You remember that in order to distinguish between the acting factors of the organizers and of the inherent potencies, we had to find conditions in which inductors and induced organs were different and that these conditions were realized in the xenoplastic experiment. The answer we obtained from this latter experiment was positive: there is actually an induction of organs which are not present on the host acting as organizer, and yet the further investigation shows that there are no specific organizers present for these foreign organs.

How then do the organizers act?

We know now that these organizers do not proceed by actions in detail and that the induction

which occurs must be of a rather general nature such as a transformation of the transplanted tissue into new regions as "upper", "lower" and "median" head region. In other words, the transplanted tissues are transformed into new areas, but we shall never witness such a thing as, for example, a specific induction into one horny tooth, or one sucker cell.

Once this transformation into a new region is realized, all the weight of the action relies upon the potencies of the reacting material and these potencies are limited by the stage of determination already reached by the donor and by its hereditary constitution.

Thus, we witness in each individual development a true creation of differentiation. Indeed, when we isolate parts of the gastrula, as Holtfreter did, by the methods of tissue culture, we bring out only the very slight inherent potencies of the egg which has already received some impulses of differentiation. In this case, the explanted parts manifest their potencies corresponding to the general pattern of organ rudiments.

But, by transplanting the same parts into the orbital cavity or into the general cavity of a larva, we submit these tissues to various and uncontrollable factors—and so they are being induced to manifest their totally surprising and bewildering tendencies of determination.

If now we transplant parts of the embryo into a definite region, this part is then submitted to very direct and precise actions of determination, thus explaining the complete reversal of the fate of the transplanted regions.

There is evidently in the living embryo something which gives to the parts of the egg an impulse to become a definite area and only then do the inherent potencies of the embryo become manifest and produce organs which belong to these regions. Induction seems chiefly to consist in an organization into regions. But there is a limit in this action of induction into new regions —sometimes the inertia of the reacting material is stronger than the inductor. This is shown by numerous cases I observed, in which the transplant was very large. As is visible on the demonstrated embryo, there is no trace of any differentiation into Anuran organs on the whole length of a very huge transplant, and yet it is visible how perfectly the Anuran skin was assimilated to the host. That a very large piece of presumptive ectoderm remains plain skin without manifesting the effects of induction into a new region is very significant. It shows that development is a very complicated result of an equilibrium between active forces which are the organizers and between the inherent potencies of the reacting material.

If we recapitulate the whole cycle of investigations carried out by the means of homeoplastic, heteroplastic and xenoplastic transplantations, we will get a clearer idea about the respective actions of the organizers and of the inherent potencies in embryonic segregation and in induction.

It is obvious that in the homeoplastic experiment performed by Spemann, the organizers and the inherent potencies are the same. It results from these experiments that the fate of organ primordiums is altered and will always correspond to the new environment.

To find out what is the part of the organizer in this action of the environment, the heteroplastic experiment was carried out by Spemann. One could legitimately presume that the strength of the organizer of one species might impose upon the donor tissue the morphological tendencies of its own species. This was proven not to be true. The result of the experiment shows indeed that the inherent potencies manifest their specific characteristics despite their submitting to the action of the foreign organizer.

In the xenoplastic experiment, at last, for the first time we were able to separate the action of organizers from the action of the inherent potencies of the donor. Indeed, Anuran and Urodele larvae possess different organs in the head region and if there should exist organizers for these different organs, their action would become manifest if brought into contact with the xenoplastic donor tissue.

The outcome of this experiment shows that organs are induced at the expense of the transplanted tissue which are different from those the organizers were supposed to form. Yet, closer analysis shows that there are no specific organizers present for these organs. The final outcome of this experiment proves to be a restriction in the action of the organizers—these latter factors producing by their intervention merely a transformation of the transplanted tissues from one region into another. It is the task left to the inherent potencies to form the necessary organs belonging to these regions independently of the nature of the host on which they are placed.

There is one question left—and that is, how does the organizer cause the induction of new regions at the expense of material coming from another region? My experiments, however, are not appropriate to give an answer to this question, exactly as the very first Spemann transplantation experiment was incapable of doing so. We are coming back then to our starting point.

In this eternal coming back of scientific problems, we are, however, happy to know that the "dead" organizer will be of great help. Freed from its ties with living material, it will allow us to

study more systematically and rationally the effects of its action.

We know the organizer is "dead"—we can buy it in a drug store, and yet is it really dead? Spemann's head and trunk organizer, and also the results of xenoplastic transplantations showing this strange induction into new regions cannot yet be explained by the mere presence of glycogen. Furthermore, would we really dare to assert, when a piece of transplant is too big and the host proves to be incapable of organizing the transplant into new regions, that there is not enough glycogen in the embryo to induce this mass of tissue?

I believe, that like the legendary Phoenix, who each time he is burned, rises again from his ashes in new splendor, the dead organizer will still give us some hard problems to solve, in order to prove to us that the phenomena of embryonic segregation and of induction cannot yet be understood by the sole action of one chemical substance.

(This article is based on a lecture presented at the Marine Biological Laboratory on August 2).

MOTION PICTURES SHOWING SOME VARIETIES OF NERVE IRRITATION, AS SEEN IN LIVING FROG TADPOLES

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The reactions of single internodal segments of myelinated nerve fibers in living frog tadpoles have been observed in minute detail following many varieties of experimental irritation of both acute and chronic types. Illustrative ciné-photomicrographic records have been obtained. Below are specified the titles of some of the pictures:

1. Acute irritation and rapid metamorphosis of a myelin segment immediately after a hot water burn. (A continuous picture showing myelin swelling, rippling, and ovoid formation.)

2. Swelling and wrinkling of the myelin sheath following administration of a strong anesthetic (chloretone).

3. The production of beaded myelin by alcohol intoxication.

4. The swelling reaction shown by the same two myelin segments photographed immediately before and immediately after alcohol treatment.

5. Nerve irritation from low temperature (tadpole embedded in packed snow for several hours).

6. Examples showing trophic (Wallerian) degeneration of a myelinated fiber after section.

7. Irritation and recovery of a myelinated fiber following adjacent wound infliction.

8. Traumatic irritation and recovery of a proximal stump myelin segment after nerve section.

9. Examples of myelin segments showing delayed irritation, several days after exposure to x-rays.

10. Chronic irritation resulting from starvation. (Several examples of wrinkled and swollen nerves after six days' total starvation.)

11. Granular degeneration of nerve fibers and sheath cells associated with prolonged inanition (20 days' starvation).

12. Effects on nerves of administration of thyroid gland extract.

13. Pressure irritation showing delicate connecting strands between the axis cylinder and myelin sheath as these two structures undergo separation.

14. Temporary irritation and recovery of a myelin segment between two wounds, the nerve fiber remaining intact.

15. Polariscopic pictures showing the behavior of the anisotropic material of the myelin sheath during various stages of growth, irritation and degeneration.

16. Degeneration of sheath cells on normal nerves.

17. Leucocytes, chiefly macrophages, moving about in a degenerating mixed nerve.

18. A macrophage leaving a blood capillary sprout (diapedesis).

19. A macrophage containing ingested anisotropic granules moving about within a single degenerating myelin segment. (Pictures taken both with ordinary light and with polarized light.)

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 1.)

THE EFFECT OF FAT SOLVENTS UPON THE FIXATION OF MITOCHONDRIA

(*Continued from Page* 193)

acids, alcohol, ether, acetone and chloroform, prevents the fixation of all mitochondria irrespective of the other constituents of the fixative, except in the case of certain complex mixtures which contain $Os\ O_4$. At present the chemical reactions involved in this type of fixation are not known.

The fixing properties of several different groups of fat-soluble substances have never been investigated, the aliphatic aldehydes, for example, Formaldehyde is the only member of this group which has been used extensively. It preserves mitochondria as slender thread-like bodies, even when mixed with the fatty acids, provided it be given a head start so that it penetrates the tissue before the acids. Acetaldehyde, propionic aldehyde and butyric aldehyde, on the contrary, not only do not fix mitochondria themselves but also prevent the fixation by such fluids as Müller's, which normally preserve them. Mixtures of picric acid and any of these aldehydes fix mitochondria, although each substance by itself does not preserve them.

The amines form a group of fat solvents, also, whose fixing properties have never been investigated. Those used in the present investigation are ethylene diamine, ethyl-, di-ethyl-, and tri-ethyl amine, di-methyl-, and tri-methyl amine, pyridine and di-iso-amyl amine. These amines cannot be used alone as fixatives for they are so alkaline that they distort the specimen to such an extent that it cannot be investigated critically. When added to a 1% solution of $H_2\ Cr\ O_4$ until it reaches pH 5.0, the mixtures form bichromates which preserve mitochondria. In this they resemble those inorganic salts of chromium previously described (Zirkle 28, 33). Mitochondria are also preserved by the mixtures at pH 6.0 but here the other components of the cell are badly distorted. When added to $K_2\ Cr_2\ O_7$ or $Cu\ Cr_2\ O_7$, these amines also improve the fixation of mitochondria. On the addition of fatty acids to the mixture, the images become acid (even at pH 5.0) and all mitochondria are destroyed.

Unlike other fat solvents, the amines do not destroy mitochondria. The question naturally arises as to the fat solubility of the compounds formed by the amines plus chromic acid. The partition coefficients of these compounds between water and ether have not yet been determined. Certain of them, such as the compound formed by di-iso-amyl amine, however, are very soluble in most non-polar solvents. In addition, a proportion of the amines remains uncombined, even at pH 5.0. When the amines are given a head start by the specimen being placed in them from 1 to 5 minutes before it is put into the fixing mixture, the mitochondria are well preserved.

At present, speculation as to the chemical composition of mitochondria is hardly justified. It is necessary first to accumulate many more facts about their solubilities.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 1.)

THE BIOLOGICAL LABORATORY
COLD SPRING HARBOR

THE INFLUENCE OF SALTS ON THE ELECTRIC CHARGE OF SURFACES IN LIQUIDS

HAROLD A. ABRAMSON AND HANS MÜLLER

There have been a good many investigations of the electrokinetic potential, ζ, of flat surfaces (or large particles) in contact with liquids. In most cases ζ is calculated from the theory of Helmholtz-Smoluchowski[1],

$$\zeta_E = \frac{4\pi\eta v}{D}, \qquad (1)$$

or

$$\zeta_S = \frac{4\pi\eta E}{DPR}, \qquad (1a)$$

where the subscript, E, refers to calculations from electrophoretic mobility v; the subscript S refers to calculations from measurements of streaming potential, E; η = coefficient of viscosity; D = dielectric constant; P = pressure; R = specific resistance. All units are c. g. s. electrostatic, and properties of the liquid in the double layer are those of the solvent in bulk. In nearly every instance the inert surfaces have a value of ζ which, in the case of electrolytes not reversing

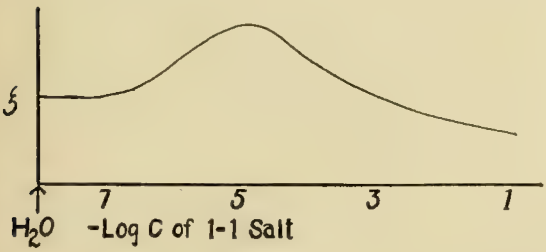

Fig. 1

the sign of charge, at first increases on addition of salt and which on subsequent addition of salt passes through a maximum (Fig. 1) then decreasing in much the same way that the electric mobility of an ion decreases as the salt concentration increases.

This decrease in ζ on the addition of salt does not by any means indicate, as has been frequently assumed, that the particles are discharged. The density of net surface charge[2] for large particles in contact with an electrolyte can be calculated for the case of an infinite plane surface from the Poisson equation:

$$\frac{d^2\phi}{dx^2} = -\frac{4\pi\rho}{D} \qquad (2)$$

where ϕ is the potential at a distance x into a liquid having a volume density of charge ρ, and the distribution of the charges in the liquid by the Boltzmann equation of ions of any valence z, of number per cm.^{3}, n,

$$\rho = (n_1 z_1 \mathbf{e})\, e^{\frac{-z_1\phi\mathbf{e}}{kT}} - (n_2 z_2 \mathbf{e})\, e^{\frac{+z_2\phi\mathbf{e}}{kT}}, \qquad (3)$$

where the subscripts 1 and 2 refer to cation and anion respectively, $\mathbf{e} = 4.77 \times 10^{-10}$ E. S. U., $k = 1.37 \times 10^{-16}$ ergs. per degree, and T is the absolute temperature.

By multiplying equation (2) by

$$\frac{\mathbf{e}}{kT}$$

and substituting for ρ we obtain, letting

$$n_1 z_1 = n_2 z_2 = NZ \text{ and } \frac{\phi\mathbf{e}}{kT} = \Psi \qquad (4)$$

$$2\frac{d^2\Psi}{dx^2} = \left(\frac{8\pi NZ\mathbf{e}^2}{DkT}\right)\left(-e^{-z_1\Psi} + e^{+z_2\Psi}\right)$$

The expression

$$\frac{8\pi NZ\mathbf{e}^2}{DkT} = \kappa^2,$$

where κ is identical with the same expression in the Debye theory of electrolytes. Letting $y = \kappa x$ and integrating once with respect to Ψ,

$$\left(\frac{d\Psi}{dy}\right)^2 = \frac{1}{z_2} e^{z_2\Psi} + \frac{1}{z_1} e^{-z_1\Psi} + A, \qquad (5)$$

When $x = 0$, $\Psi = 0$ and $\dfrac{d\Psi}{dy} = 0$,

so that, (6)

$$A = \frac{1}{z_1} - \frac{1}{z_2}\,.$$

Combining equations (5) and (6), and substituting $N = 6.06 \times 10^{23}$ molecules per gm. mole, c = concentration in moles per liter and putting

$$\frac{d\Psi}{dy} = \frac{4\pi\sigma e}{DkT\kappa}$$

where σ is the *surface* density of charge we obtain σ as a function of the potential, ζ, of the plane surface, and of the concentrations c, with all the other teams constant or easily measureable,

$$\sigma = \sqrt{\frac{NDkT}{2000\pi}\, c_{1(2)}\, z_{1(2)}\left[\frac{1}{z_2}\left(e^{\frac{z_2\zeta e}{kT}} - 1\right) + \frac{1}{z_1}\left(e^{\frac{-z_1\zeta e}{kT}} - 1\right)\right]}\,. \quad (7)$$

Note that equation (7) is valid for ions of any valence and holds independent of the value of

$$\frac{\zeta e}{kT}$$

which must be small in the Debye theory for particles having small radii. In the case $z_1 = z_2$ equation (7) reduces to

$$\sigma = 2\sqrt{\frac{NDkT}{2000\pi}}\,\sqrt{C}\,\sinh\frac{z\zeta e}{kT}\,. \quad (8)$$

The use of equation (8) for uni-univalent salts in aqueous solution is simple for it may be written when $z_1 = z_2 = 1$

$$\sigma = 2\,\alpha\sqrt{C}\,\sinh\frac{\zeta}{\beta} \quad (8a)$$

$\alpha = 17{,}650$ and $\beta = 0.025$ volts at 18°, assuming the dielectric constant to be that of the solvent.

Uni-univalent Salts

There is much experimental work in the literature which, for example, gives data for ζ particularly on glass[3], quartz[4], paraffin oil[5], cellulose[6], clay[7], graphite[8] and collodion[9]. By means of equation (8) the variation in σ has been plotted from these data as a function of the added salt concentration in Figures 2-10. (Correcting for the concentration of ionized H_2CO_3). Note the regularity in all of the curves. In each instance regardless of the nature of the material σ increases sharply and almost linearly at low concentrations rapidly changing the rate of charge, reaching a limiting value at about c = 0.01 M. The general shape of the curve is the same for all of the data on surfaces of this type known to the authors.

It is evident that the descending part of the ζ -c curve given in Fig. 1 instead of representing a discharge of the particles actually is accompanied by an *increasing* charge. It is unfortunate that no data are available in stronger uni-univalent salt solutions where σ conceivably might decrease. The complicated course of the ζ-c curve is due to the fact that ζ depends upon two variables, σ and κ, both of which depend upon c. We have here done what is tantamount to assuming the validity of the theory of the diffuse double layer to obtain the dependence of κ on c, permitting the calculation of σ (c). σ and ζ presumably vary quite differently with c. As σ increases $1/\kappa$ decreases. When c is small the increase in σ is more important, but as c increases the decrease in $1/\kappa$ becomes more important flattening out the σ -c curve[2].

It will have been noted by the reader that the form of all of the σ -c curves bears a marked resemblance to the Langmuir adsorption isotherm. The smooth curves of Figures 3, 4 and 5 etc., have actually been plotted by an equation of the form

$$\sigma = \sigma_L \frac{\beta C}{1 + \beta C} \quad (9)$$

which is for the simplest case of adsorption of one type of particle, where $\sigma_L\,\beta$ is the initial slope of the σ -c curve and σ_L is equal to the limiting value of σ within the concentration range. The agreement is very good indeed and indicates that selective adsorption of the negative ion takes place. The curves for the different alkali halogens and alkali earth halogens differ much less than the difference between the curves for potassium bromide, iodide and chloride. Especially large values of the charge are reached for the sulfides and ferro cyanides. The data indicate that for glass, graphite and col-

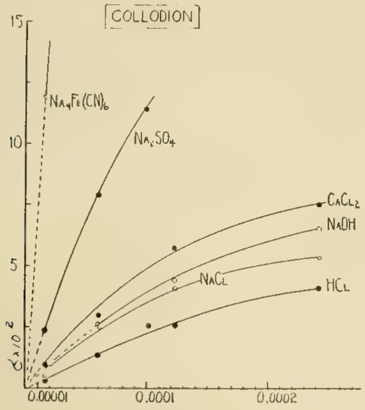

Fig. 2a

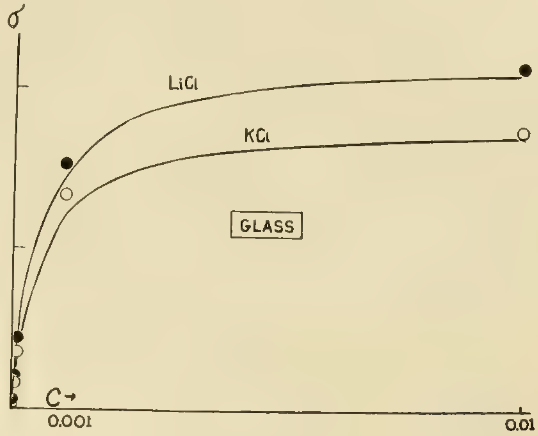

Fig. 3

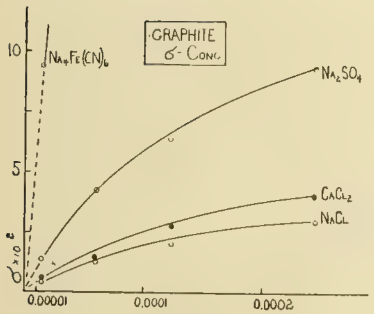

Fig. 2b

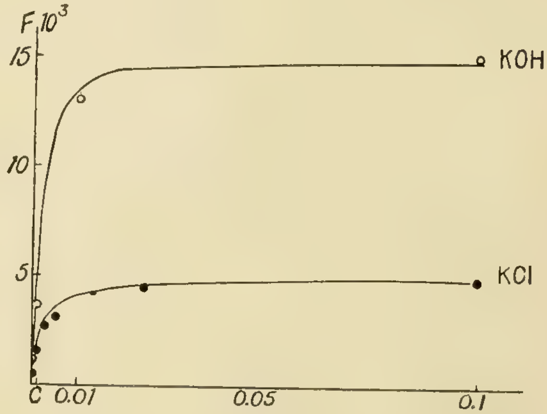

Fig. 4

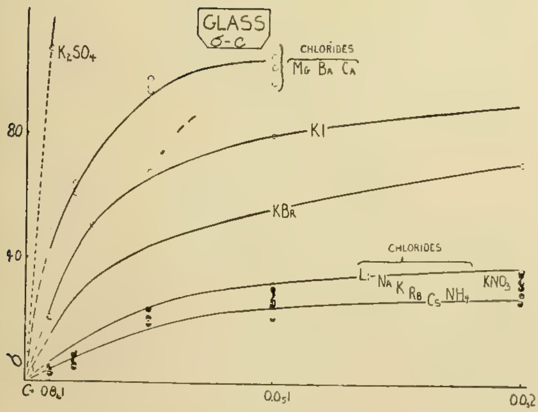

Fig. 2c

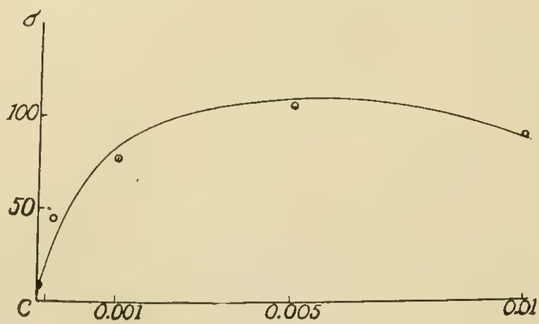

Fig. 5

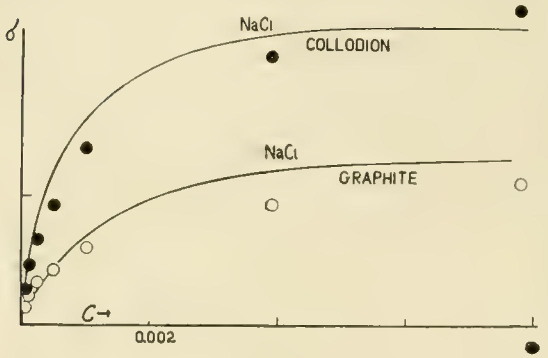

Fig. 6

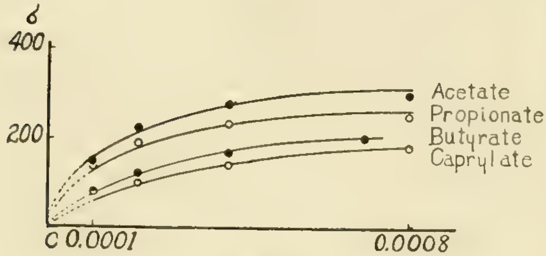

Fig. 7

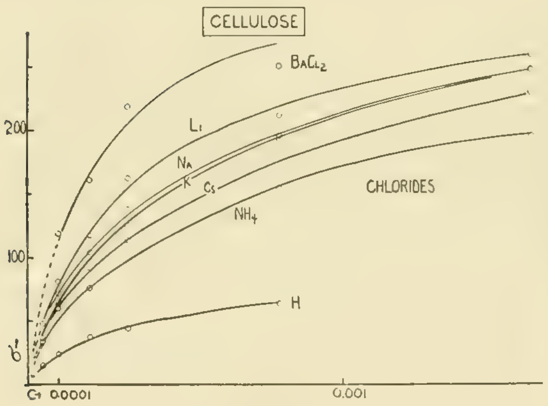

Fig. 8

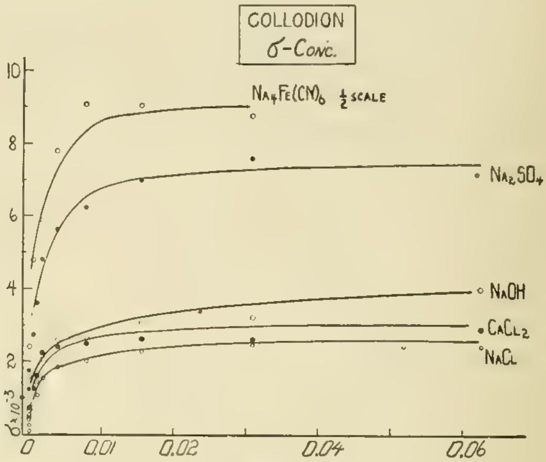

Fig. 9

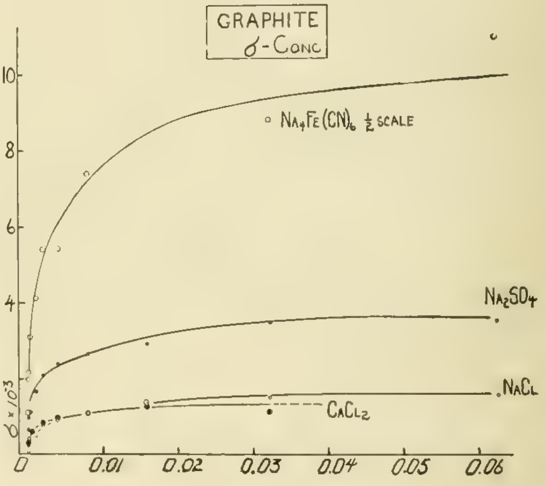

Fig. 10

lodion the adsorption potential increases in the series:

Cl^-, Br^-, I^-;
Li^+, Na^+, K^+, Rb^+, Cs^+;
Mg^{++}, Ba^{++}, Ca^{++};
I^-, $(SO_4^{=}$ $[Fe(CN)_6]^{\equiv}$

and that in general for these surfaces the adsorption potential of the positive ions are considerably smaller than those of the negative ions.

The initial slope of the σ (c) curves leads to values of the adsorption potentials of the same order of magnitude as found for the adsorption of gases.

It is of interest to calculate the fraction of the area occupied by ions accounting for the limiting value of σ. To take the case of glass, σ_L = 9000 E. S. U., representing about 2×10^{13} ions per cm^2. If the area occupied by each ion is taken as 5×10^{-16} cm^2, the area occupied by the ions given by σ_L is 0.01 cm^2 or 1% of the surface. This is a rather higher value than that deduced by previous investigators, but is compatible with the type of adsorption postulated by Langmuir's theory.

Polyvalent Ions

In a future communication we shall give results of calculations by means of equation (7) for the σ -c curve for non-symmetrical cases of polyvalent ions not reversing the sign of charge. Using the Debye approximation for surfaces where $\frac{\zeta e}{kT}$ is relatively small, we have obtained preliminary indications that the form of the curve is exactly the same as that obtained by the rigorous expression (equation 8) for uni-univalent salts. Some of the curves are given in Figs. 9 and 10 (collodion and graphite). Compare these curves with the σ -c curves for the same substances using the full equation. There is obviously no difference in the shape of the curves.

In previous communications the authors have shown that for certain inorganic solutions[10] and protein solutions[11] the effect of salts on ζ could be explained by the effect of salt on κ, with σ taken as constant. The group of substances here studied are more complicated in that both σ and κ depend more markedly on the salt. They all, nevertheless, show the remarkably constant shape of the σ -c curve which can probably be taken as generally true for inert surfaces in solutions of salts not reversing the sign of charge.

Summary

It is proposed that an advance in the study of the constitution of the solid-liquid and liquid-liquid interfaces may be made by calculation of the surface density of electric charge σ, in addition to the analysis of the potential difference, ζ, between the movable phases. This calculation has been made for the negatively charged surfaces of graphite, glass, quartz, cellulose, "collodion" and paraffin oil, in the case of ions (valence 1:1; 1:2; 2:1; 1:4) not producing reversal of sign of charge. Although the ζ — concentration curves are rather complex, the σ — concentration curve in every case yields a simple curve resembling typical adsorption with an initial steep slope and saturation at about 0.01 Molar. The character of the curves is determined by both ions. The slope at zero concentration varies markedly, particularly with value of the anion; e. g. for glass in the order, I > Br > Cl, the differences diminishing as saturation is approached. The method of calculation employed here to ascertain the nature and magnitude of the forces involved in adsorption of ions by "inert" surfaces at the liquid interface is justified by previous theory and experiment of the authors.

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Discussion

Discussion of this paper is included in that of Dr. Müller's paper, "The Theory of Ionic Adsorption."

THE THEORY OF IONIC ADSORPTION

HANS MÜLLER

In the paper on the theory of the diffuse double layer it was pointed out that the existence of interphasial potentials leads necessarily to the conclusion, that the surfaces are the seat of electric charges. There are three possibilities to explain the origins of these charges. The first possibility can only play a rôle if both phases are electrolytic conductors. The accumulation of the charges is then due to the difference of the ion activities in the two phases. If one phase is a solid, this possibility is eliminated. A solid phase can acquire the charge by dissociation. If the material has the tendency for dissociation, it will send ions of one kind into the solution, and the charge is due to the ions or electrons whose charge is otherwise neutralized by the dissociated ions. The third possibility is adsorption. In this case the solid does not send ions into the solution, but its surface attracts electrolytic ions. They are bound to the surface by the unsaturated valencies of the atoms on the surface, or by the Van der Waals forces of the molecules of the solid. In general two or all three effects may be responsible for the surface charges. It is, therefore, in most cases, not possible to decide whether we are dealing with dissociation or adsorption.

The theory of the diffuse double layer offers a means of calculating the surface charge σ. In the first paper the following relation was derived:

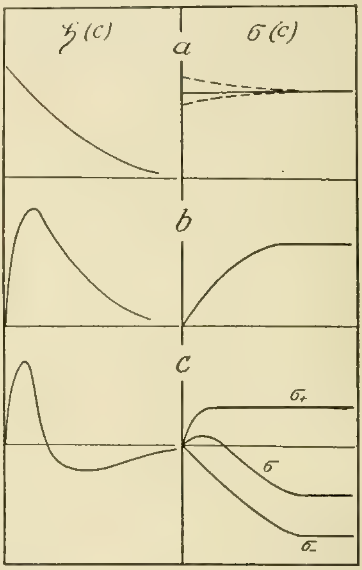
$$\sigma = \sqrt{\frac{DKT}{2\pi} \Sigma n_i^o \left(e^{-z_i \frac{e\zeta}{kT}} - 1 \right)} \qquad (1)$$

This formula permits one to calculate the charge per cm.^{2} from the measured electrokinetic potentials ζ, the concentration n_i^o, and the valencie z_i, of the ions in the electrolyte.

The observations on the change of the ζ potential with the electrolyte concentration c, give, for different surfaces and different solutions, a great variety of ζ (c) curves, and it seems, at first glance, to be quite impossible to recognize any kind of regularity. In general the ζ (c) curves can be classified into three groups, which shall be discussed successively.

Group a.

Very frequently the ζ (c) curves (Fig. 1, a) start for very low concentration with a relatively large value of ζ and decrease continuously with increasing concentrations. This is, for instance, the case in the measurements of Freundlich and Zeh [1] on As_2S_3 and Fe_2O_3 colloids. Assuming a reasonable value for the radius of the micelles, I was able to show [2] that these curves can be explained without assuming any change of the charge σ. The decrease of the ζ (c) curve is only due to the change of the thickness of the double layer. Even if the actual radius of the particles (which is not known) would have been



The three types of ζ (c) curves and the corresponding σ (c) curves.

twice as large, or half as large, the data would have given a change of σ by less than 10%. Similar verifications of this fact were given by Paine [3]. We are, therefore, justified in assuming that in these cases no appreciable change of charge takes place. These examples furnish the best verification of the theory of the diffuse double layer. They justify the use of this theory for the more complicated cases.

A similar course of the ζ (c) curves is observed with the proteins; here, in general, they pass through the Zero axis. There exists an isoelectric point, where the ζ potential changes its sign. The isoelectric point is, however, not determined by the electrolyte concentration, but depends primarily on the hydrogen ion concentration. Also here there is no adsorption. The charge of the protein particles is determined by the dissociation equilibrium of the H and OH ions. The elec-

trolyte changes the charge only insofar as it produces a slight change of this equilibrium.

Group b.

The ζ (c) curves of this group (Fig. 1, b) start with a small value of ζ for very low concentration. They increase first very rapidly and reach a maximum for concentrations of about 10-100 micromol per liter. For still higher concentration they decrease again in a way similar to the curves in Group *a*. It is probable that many earlier investigations which gave ζ (c) curves of Group *a* belong really in Group *b* since their failure to show the initial increase is only due to the fact that the colloids were not satisfactorily dyalized.

This complicated course of the ζ (c) curves can, in my opinion, only be understood if one realizes that the ζ potential is determined by two factors, namely, the charge of the double layer, and its thickness. If we accept the theory of the diffuse double layer, we can calculate the thickness of the double layer, and we are, therefore, able to calculate the charge σ and its variation with the help of formula (1). Such calculations were recently carried out by Abramson, and are reported in the preceding paper. The following discussion refers to the figures of this paper. All ζ (c) curves show a linear increase of the charge σ with small concentrations. For large concentrations the charge tends towards a saturation value. While the initial slopes of these curves and their saturation values change with the material of the solid phase and the electrolyte, the type of the curves is the same in all cases studied. Before we begin the discussion of these results, I wish to describe briefly the course of the most general ζ (c) curves.

Group c.

The ζ (c) curves of this group start the same way as those of Group *b* (Fig. 1. c). But, after reaching their maximum, they decrease faster, pass through an isoelectric point, and reach a minimum, which is usually much smaller and flatter than the first maximum. For still higher concentration they approach again the value $\zeta = 0$. To my knowledge, no calculations of the σ values for this group of ζ (c) curves have been calculated. It is easy to realize what the course of the σ (c) must be. They will start (Fig. 1, c) at $\sigma = 0$ and reach a maximum for about the same concentration where ζ reaches its first maximum. At the isoelectric point we must have $\sigma = 0$ and for higher concentrations the charge will approach a saturation value of a sign opposite to the sign of the charge for small concentrations.

The σ (c) curves calculated by Abramson are very similar to the well known adsorption curves of Langmuir. It seems, therefore, that we are dealing here with adsorption, and that we are justified in using Langmuir's theory of adsorption. Gyemant[4], and Stern[5] have repeatedly suggested that this theory may be applied to ionic adsorption. Abramson's data lead to the same conclusion.

The laws of adsorption can be derived by the following consideration. The adsorption is due to forces exerted by the surface. These are molecular forces. They fall off very rapidly with the distance from the surface. Their range of action is of molecular dimension. To simplify the calculation, one assumes that the adsorption range has a definite thickness d and that a molecule or ion within the adsorped layer has an average potential energy (-A). According to Maxwell-Boltzmann's principle the number n_a of molecules per cm.³ in the adsorption range is then

$$n_a = n\, e^{\frac{A}{kT}}$$

Where n is the number of molecules in 1cc in the immediate neighborhood of the adsorption range. If we are dealing with ions, the number of ions just outside of the adsorption range differs from their number in the electrolyte. This is due to the electrostatic forces in the diffuse double layer. We have

$$n = n^o\, e^{-z\frac{e\zeta}{kT}}$$

and hence

$$n_a = n^o\, e^{\frac{A - z e \zeta}{kT}}$$

Since the adsorption range has a thickness d, the number of adsorbed ions per cm.² is then $n_a \cdot d$, and the adsorbed charge

$$\sigma = n_a\, d\, z\, e$$

We assume that this consideration holds for each kind of ions. The adsorption potential A will, of course, depend not only on the nature of the surface, but also on the nature of the adsorped ion. We consider here the case where the electrolyte contains one kind of anions and cations of for valency z_1 and z_2 respectively.

For small concentrations we have then

$$\sigma = n_o\, e\, d\, z_1\, z_2 \left(e^{\frac{A_1 - z_1 e \zeta}{kT}} - e^{\frac{A_2 + z_2 e \zeta}{kT}} \right)$$

where A_1 and A_2 are the adsorption potentials of the positive and negative ions respectively.

For small concentrations σ and ζ are small, and we can develop

$$\sigma = n_0\, e\, d\, z_1 z_2 \left(e^{\frac{A_1}{kT}} - e^{\frac{A_2}{kT}} \right) - n_0\, e\, d\, z_1 z_2 \frac{e\zeta}{kT} \left(z_1 e^{\frac{A_1}{kT}} + z_2 e^{\frac{A_2}{kT}} \right)$$

For small values of ζ we have, however,

$$\zeta = \frac{4\pi\sigma}{D} \frac{1}{\kappa}$$

where

$$\kappa^2 = \frac{4\pi n_0 e^2}{D\,k\,T} z_1 z_2 (z_1 + z_2)$$

and hence

$$\sigma = \frac{n_0\, e\, d\, z_1 z_2 \left(e^{\frac{A_1}{kT}} - e^{\frac{A_2}{kT}} \right)}{1 + \frac{1}{z_1 z_2} \kappa d \left(z_1 e^{\frac{A_1}{kT}} + z_2 e^{\frac{A_2}{kT}} \right)}$$

$1/\kappa$ is the thickness of the double layer. For small concentration $1/\kappa = \lambda$ is about 1000 times larger than d. Hence the denominator is practically equal to unity, and we have

$$\sigma = n_0\, e\, d\, z_1 z_2 \left(e^{\frac{A_1}{kT}} - e^{\frac{A_2}{kT}} \right) \qquad (2)$$

This equation states that for small concentrations the surface charge is proportional to the concentration. If the adsorption potentials A_1 and A_2 are markedly different, then σ will have the sign of the stronger adsorped ion. Introducing this value of σ in the equation of the double layer gives, for small concentration, a linear relation between ζ and $\sqrt{C}$.

The data on glass, graphite and collodion, Figs. 2 to 8 of the preceding paper by Abramson and Müller, demonstrate the validity of this formula. Since all these surfaces are negatively charged, the halogen ions must have the higher adsorption potential. Consequently, the differences between the various alkali chlorides, and between the earthalkali chlorides, are much smaller than between potassium-chloride, bromide and jodide. The valency z_1 of the positive ion is responsible for the fact that the earthalkali chlorides give a greater slope than the alkali chlorides, and accounts partly for the large charging action of the sulfides and ferrocyanides. The σ (c) for low concentrations are not reliable enough to give more than a qualitative check of the theory. This is due to the fact that for these concentrations the formula for the electrophoretic migration speed is uncertain. The order of magnitude of the adsorbed charges is, however, quite reasonable. For KBr on glass we have, for instance, an initial slope

$$\frac{d\sigma}{dc} \cong 10^7 = \epsilon\, d \left(e^{\frac{A_1}{kT}} - e^{\frac{A_2}{kT}} \right) 6 \cdot 10^{20}$$

which gives with $d = 3\ 10^{-8}$

$$e^{\frac{A_1}{kT}} - e^{\frac{A_2}{kT}} = 10^5$$

leading to adsorption potentials of the order of magnitude of 10 k T. Langmuir's data on the adsorption of molecules give values of A of the same magnitude(6). The data on glass give some interesting information concerning the dependence of the adsorption potential on the nature of the ion. Since the curve for KI is higher than the one for KBr, and since this curve is again steeper than the one for KCl, we conclude that the adsorption potential of the halogen ion increases in the series Cl, Br, I. Similarily we conclude that the adsorption potential increases in the series of ions, Li, Na, K, Rb, Cs, and Mg, Ba, Ca. Further data are required for a decision as to whether or not these series hold for any kind of surfaces. The σ (c) curves for collodion and graphite indicate that this might be the case. In agreement with the data on glass, they give a very much larger adsorption potential for SO_4 than for Cl (in $CaCl_2$), and a still larger value for $Na_4Fe(CN)_6$. The data even indicate a constant ratio of the initial slopes. The figures give approximately the following values of

$$\frac{d\sigma}{dc} \cdot 10^{-6} \text{ for small concentrations.}$$

Electrolyte	Collodion	Graphite
$NaCl$	4.0	2.4
$CaCl_2$	5.2	3.0
Na_2SO_4	13.5	8.0

The values for collodion are in all three cases about 70% higher than for graphite. This may be due to the fact that collodion has a wider adsorption range, or that all adsorption potentials on collodion are larger by the same amount than the potentials on graphite.

The very limited amount of reliable data available does not permit any definite conclusions. The above discussion will merely indicate how the measurements could furnish important information concerning the adsorption potentials and the range of the adsorption forces.

For higher concentrations we have to take into account the fact that only a limited number of ions can be adsorped. Langmuir found that the entire course of the adsorption curve can be represented by the formula

$$n_a = \frac{\alpha n}{1 + \beta n}$$

To satisfy the limiting case of small concentrations we must have

$$\alpha n = d\, n_0\, e^{\frac{A - z e \zeta}{k T}}$$

The saturation value of the number of adsorbed atoms is then α / β. This value shall be denoted by S, and we have

$$n_a = \frac{n_0\, d\, e^{\frac{A - z e \zeta}{k T}}}{1 + \frac{n_0\, d}{S}\, e^{\frac{A - z e \zeta}{k T}}}$$

Gyemant and Stern have proposed that such an adsorption isotherm may be used for both kinds of ions. It is doubtful whether this is justified. Very little is known concerning the simultaneous adsorption of more than one kind of molecule. The data available point strongly to the fact that we have no superposition of the two adsorption curves, but that there is "selective" adsorption. One kind of molecule is preferred, and prevents the adsorption of the other kind of molecule. The well known exchange adsorption in colloids indicate that similar effects take place in ionic adsorption.

Independent of the exact law, saturation will always occur, as is observed in the σ (c) curves. The saturation value of the charge is given by

$$\sigma_{max} = e\,(S_1 z_1 - S_2 z_2)$$

The observed values of σ_{max} vary between 100 and a few thousand E. S. U. Using $\sigma_{max} = 1000$ gives $(S_1 z_1 - S_2 z_2) = 5\ 10^{12}$. Since the ionic radii are of the order of 10^{-8} cm., S_1 and S_2 could have values up to 10^{15} without contradicting the assumption that the adsorbed layer is monomolecular.

The Langmuir theory is, therefore, in principle quite sufficient for the explanation of the σ (c) curves of Group 2. The more complicated σ (c) curves of Group *c* could be explained by assuming that a Langmuir isotherm holds for each kind of ion, and that we have

$$A_1 > A_2$$
$$S_1 < S_2$$

Two such isotherms (Fig. 1,c) would indeed result in a σ (c) curve of the type required, but it is questionable whether the two above conditions are compatible.

So far it was assumed that the entire charge σ, calculated from the ζ (c) curves, is adsorbed. Actually this value of σ is the charge within the rigidity boundary. We have seen that this boundary does not necessarily coincide with the surface of the adsorption range. There exists, therefore, the possibility that the calculated σ must be considered as the difference of two charges, namely the real adsorbed charge and the charge between adsorption range and rigidity boundary. It is evident that such an assumption introduces many uncontrollable factors and opens the possibility to explain almost any ζ (c) curve. O. Stern has given a theory of this type. He assumes a rigid double layer within the rigidity boundary, the outer layer of which does not entirely compensate the adsorbed charges. The compensation is completed by the diffuse double layer. Stern's picture of the charge distribution in a surface has the great advantage of being able to explain satisfactorily the difference between ϵ and ζ potentials, the existence of isoelectric points and the experimental values of the electric capacity of the double layer, but it introduces so many variables, that it is quite impossible to decide whether or not the assumptions are correct.

Before these questions can be satisfactorily settled, it is necessary to have many more reliable measurements of ζ (c) curves on well defined surfaces, using a large number of different electrolytes.

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Discussion

Dr. Shedlovsky: Has there been any attempt to deal with the question of adsorption on crystalline surfaces? The crystallographic periodicity might produce periodicities of the adsorption potential and modify the charge density, particularly if the size of the adsorbed ions is smaller, or of the same magnitude, as the lattice constant.

Dr. Müller: Hückel has given a discussion of this question, but no experimental data are available which could verify his conclusions.

Dr. Shedlovsky: Is there a large variation of the adsorption potentials for the different ions? Is there any indication that complicated ions, which might have dipolar characteristics, show a larger effect?

Dr. Abramson: The adsorption potentials, as determined from the initial slope of the σ (c) curves, vary considerably. Accurate values are difficult to determine, because these data are to be obtained from measurements in solutions so dilute that one has to know exactly the carbon dioxide content of the water, and one must take into consideration the ionic strength of the water.

Dr. Cohen: Is there anything that can be added with reference to adsorption of ions on metallic electrodes, such as platinum or gold?

Dr. Shedlovsky: There is some work by Frumkin on the adsorption of hydrogen ions on a platinum surface. If the solution is saturated with hydrogen, practically no adsorption of hydrogen ion on platinum is evident.

Dr. Cohen: I was referring to blank metal.

Dr. Shedlovsky: The same would hold there. Electrically there seems to be very little reason to suppose that the effect of a platinized electrode is more than to provide a very large surface.

Dr. Cohen: It is perhaps also a matter of the crystal configuration of the surface.

Dr. Mudd: Is there a known physical explanation of the fact that the forces of adsorption of ions seems to be of the same order as those of uncharged molecules?

Dr. Müller: The adsorption forces are close range forces; according to the interpretation of Debye they depend on the electric dipole—or the quadrupole—moments of the molecules, and should, therefore, be of the same magnitude for charged, or uncharged, atoms.

Dr. Shedlovsky: The adsorption of ions differs only insofar as the electric double layer forms an electrostatic screen, keeping one kind of ion away from the surface.

Dr. Cole: Is there any possibility that the Van der Waals forces might act on the ions in the diffuse double layer?

Dr. Müller: This is very unlikely. The range of the adsorption forces is usually not larger than the diameter of the molecules. There are, however, cases where the adsorped layer is definitely not monomolecular. In Stern's theory, the ions, in the outer layer of the Helmholtz double layer, are held by Van der Waals forces.

THE ELECTRICAL BEHAVIOR OF LARGE PLANT CELLS[1]

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The electrical phenomena observed in living cells might conceivably arise from phase boundary potentials, Donnan constraints, oxidation and reduction, or diffusion. Recent work indicates that phase boundary potentials are usually small and cannot at present be satisfactorily measured or calculated. Potentials in living tissues due to Donnan constraints are usually small, owing to relatively low concentrations of indiffusible ions, and can be calculated only at equilibrium, which seldom or never occurs in living, growing cells. Oxidation-reduction potentials could manifest themselves if metallic electrodes were brought into direct contact with the reactants (*e. g.* by insertion into the living cell), but it is difficult to see how they could manifest themselves in the experiments here discussed[2] where contact with the organism is made by means of salt bridges. Studies of *Nitella* show that the most satisfactory calculations result when diffusion potentials alone are taken into account.

This statement concerns only thermodynamic potentials to which the present discussion is limited.

These potential differences seem to reside mainly in the non-aqueous surface layers of the protoplasm. Diffusion potential in the aqueous phases seems to be unimportant compared with that in the non-aqueous layers at whose surfaces, moreover, phase boundary potentials may occur.

These surface layers claim a special interest. They regulate the exit and entrance of materials and so determine metabolism. Their electrical behavior is a most delicate index of the condition and activities of the cell. Injury and death are indicated in this way long before any visible change occurs in the cell. Recovery from injury can be traced as in no other way. The effect of all sorts of external agents can be followed by electrical measurements which in no way disturb the vital processes. This also applies to spontaneous changes, such as action currents. Thus a fruitful field of investigation is open to the biologist.

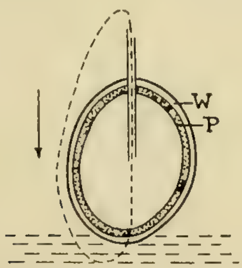

Fig. 1. Diagram of a cell of Valonia which is pierced by a glass capillary so that the protoplasm adheres to the glass and forms an electrical seal which prevents a short circuit through the cell wall. The arrow shows the direction in which the positive current tends to flow when cell sap is applied to the exterior of the cell. W, cell wall; P, protoplasm.

Large cells are especially suitable for such studies. This is particularly true of certain multinucleate cells such as those of the marine plants *Valonia* and *Halicystis* and of the fresh-water plants *Nitella* and *Chara*. They can be obtained singly and are, therefore, free from certain complications attaching to bundles of cells, such as are found in muscle and nerve.

In such cells the protoplasm appears to consist of an aqueous layer having an outer non-aqueous surface (next to the cell wall) and an inner non-aqueous surface (next to the large central vacuole, which is filled with a clear watery cell sap).

The non-aqueous surface layers are unlike. In order to show this we need only pierce the cell with a capillary (Fig. 1) and lead off from the interior to the outside which is in contact with sap extracted from another cell. We then have the chain

$$\text{sap}\ \Big|\ \overbrace{X\quad W\quad Y}^{\text{protoplasm}}\ \Big|\ \text{sap}$$

in which W is the aqueous layer of protoplasm, X and Y are the outer and inner non-aqueous surface layers. We should find no E. M. F. if X and Y were identical but this is never the case. In *Valonia* the observed E. M. F. is about 60 millivolts, in *Nitella* 15 millivolts (in both cases inwardly directed); in *Halicystis* it is about 50 millivolts (outwardly directed).

It may be remarked that other evidence shows that X and Y are different, *e. g.* X secretes a cellulose wall but Y does not. One might be inclined to say that this can hardly be due to the difference between the internal and external solutions since in *Halicystis* these are almost identical. But we must remember that the sap is more acid, contains less oxygen, and more CO_2 and organic matter than the external solution.

What is the nature of the surface layers? They appear to be liquid since they round up in contact with aqueous solutions and show true surface tension. In respect to solubility they appear to act very much like chloroform toward dyes, and more like guaiacol in relation to such substances as potassium, sodium, magnesium and calcium. In

the case of *Valonia* the potassium has apparently a greater partition coefficient in the surface layer than does sodium; those of calcium and magnesium are much less than that of sodium. Furthermore that of sulfate is much less than that of chloride. All of this applies also to guaiacol.

The apparent mobility of the potassium ion in the surface layer is much greater than that of sodium: this is especially clear in the case of *Halicystis* where the conditions are not complicated by differences in partition coefficients. Thanks to the unpublished work of physical chemists we now know that K^+ has a higher mobility than Na^+ in guaiacol which makes another point of resemblance to the protoplasmic surface.

Let us now try to picture the bioelectrical situation in *Nitella*. This may be done by means of a model[3]. Since the underlying principles can be illustrated as well by one non-aqueous layer as by two we employ a model consisting of a single non-aqueous layer *B*, with an exterior aqueous phase *A*, and an interior phase *C*, which may be called artificial sap. In our experiments *B* consisted of a mixture of guaiacol and *p*-cresol.

When KOH is placed outside and CO_2 is bubbled inside (in *C*), to imitate the production of CO_2 by the cell, potassium enters and combines in *C* to form $KHCO_3$: this reaction keeps the ionic activity product (K) (OH) lower inside than that outside so that potassium continues to enter until its concentration becomes much greater than outside. The osmotic pressure in *C* rises, and water enters. Eventually a steady state is reached in which water and electrolyte enter in the same ratio, and *C* increases in volume while its composition remains approximately constant. This seems to be analogous to what happens in living cells.

When this has happened we find an outwardly directed E. M. F. of about 40 millivolts. This is evidently due to $KHCO_3$ in the artificial sap and may be most conveniently explained on the ground that K^+ has a higher mobility than HCO_3^- or the guaiacol ion which can be proved experimentally (if phase boundary potentials enter in we may neglect them for the time being).

It is clear that potassium enters and produces an outwardly directed E. M. F. against which it continues to enter. In other words it penetrates against a constantly increasing potential gradient created by itself. We find, indeed, that at the start the E. M. F. is directed inward and as potassium enters this is reversed.

All this may be explained by saying that potassium enters in one form and leaves in another and that it sets up less P. D. in entering the artificial cell than in leaving it. For example, if the cation and anion of the entering salt had the same mobility in the non-aqueous layer it would set up no diffusion potential in this layer.

In this way we may explain the fact that *Nitella* has an outwardly directed P. D. of a hundred millivolts or more. The non-aqueous layers are probably only a few molecules thick but supposing that taken together they are a micron in thickness, we have a potential drop of 100 millivolts or more across 1 micron, equivalent to 100 volts across a layer 1 millimeter in thickness.

It thus appears that the electrical forces in the cell are of considerable magnitude. What do they accomplish?

Evidently there will be no flow of current as long as the P. D. is the same at all points on the surface of the cell. But if at any point local differences of metabolism alter the concentrations of ions and so change the P. D., there may be a flow of current between this and neighboring points and this may be accompanied by a flow of water[4].

If there be an outwardly directed P. D. of 100 millivolts or more it will give a corresponding current of injury. This can be measured by killing one spot on the cell, *i. e.* by substituting an injured area for the capillary shown in Fig. 1. Since the P. D. is outwardly directed the current flows in the external circuit toward the injured spot, *i. e.* the current of injury is negative. This is stated in the literature as the invariable situation, but this is an error. When we apply sap or 0.05 M KCl to *Nitella* the P. D. becomes inwardly directed and the current of injury becomes positive. The same can be done in *Halicystis* by applying NH_3 (Blinks). In *Valonia* it is normally positive.

When we reduce the P. D. at *A* (Fig. 2) by killing or by applying 0.01 M KCl, current begins to flow from surrounding regions to this spot. Suppose that with the outgoing current at *B* potassium moves outward from the sap until its concentration becomes as great outside as inside. The P. D. will then fall to zero. Current will then begin to flow from *C* to *B*, along the cell wall and inwards to the sap, carrying potassium back into the sap at *B*. This movement of potassium will be assisted by the forces which normally cause potassium to diffuse into the sap

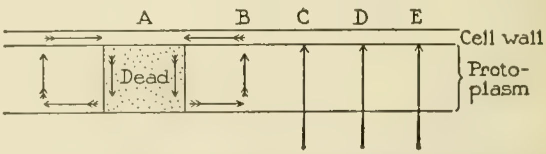

Fig. 2. Hypothetical diagram. The plain arrows denote P. D.: the feathered arrows show actual flow of current.

and accumulate when no current is flowing. As a result the potassium will resume its normal condition and the normal outwardly directed P. D. will return: this is called recovery. In the meantime the outward current at *C* will cause its P. D. to fall to zero and this will be followed by recovery. The same thing will then happen at *D* and later at *E*. (On this basis an outward electric current at *B* due to an external source should stimulate, as is actually the case.)

In this way we can explain the action current and its propagation along the cell. There is considerable evidence in favor of this view but it must be regarded as merely a working hypothesis. A different explanation is offered by Blinks, based on his finding that internal changes of pH value greatly affect the P. D. of *Halcystis*(5). He suggests that sudden increase of alkalinity in the cell caused by outward current flow, may cause a lowering of the P. D. and that recovery is due to subsequent production of acid(6). This is in harmony with the theory of Bethe.

These hypotheses are mentioned because they grow directly out of our experiments on large plant cells. Other explanations are possible but need not be discussed here.

Action currents may be produced in *Nitella*, as in nerve and muscle, by electrical or chemical stimulation. Negative variations produced by mechanical stimulation are different since they travel much faster and can pass a killed spot by the transmission of a wave of compression.

Ordinary action currents cannot pass a killed spot except by the aid of a salt bridge as shown in Fig. 3. When a propagated variation coming from *A* reaches *B* the P. D. at *B* goes to zero and in consequence current flows from *D* through the salt bridge to *B*: this reduces the P. D. at *D* to zero and in consequence current flows from *E* to *D* and by a continuation of such processes the negative variation is propagated.

When stimulated electrically *Nitella* responds like a skeletal muscle, giving a single action current: but when an area of the cell has its P. D. reduced for a period of minutes by applying 0.01 M KCl it gives a series of rhythmical responses like a heart muscle. This is to be expected on theoretical grounds.

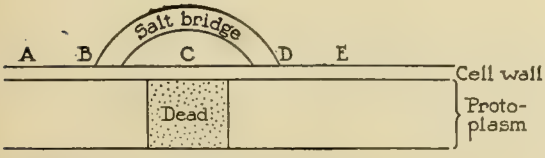

Fig. 3. Arrangement of salt bridge to enable an action current to pass a dead spot.

There is one very interesting feature of the negative variation which was described by Blinks. When the P. D. has fallen to zero the protoplasm no longer shows polarization, *i. e.* it temporarily builds up no back E. M. F. when a current is sent through it from an external source. It seems to act as though it were completely permeable to ions. The polarizability is regained as the P. D. returns.

It is of interest to find that we can apparently remove certain substances from the non-aqueous surface layers and thereby change their electrical behavior(7). After leaching with distilled water, or with acid or alkaline solutions, the cell can no longer be stimulated electrically but the irritability is restored when the cell is replaced in solutions containing calcium. Before treatment we find that potassium is very negative to sodium in *Nitella* but after treatment this is no longer the case. Evidently a marked modification of the properties of the surface has occurred.

These experiments seem to show two things (*a*) that the non-aqueous surface is a mixture of substances, some of which can be taken away and still leave a non-aqueous layer, and (*b*) that anesthesia can be produced by removing certain substances from the cell.

In conclusion it may be well to emphasize that electrical studies enable us to follow minute and rapid changes in the cell with little or no alteration of normal functions. This is the more important since most attempts to investigate life processes introduce disturbing factors of which we are often unaware.

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Discussion

Dr. Blinks: There is a point which possibly bears on the theory of the bioelectric potentials, namely, the amount of current which can be supplied by a living cell. When the circuit through a *Halicystis* cell is completed through a galvanometer, a current of 5 to 10 micro-amperes will continue to flow for several days without greatly reducing the value of the potential difference,

which remains close to 68 millivolts during this time. It seems unlikely that a phase boundary potential could supply such a current.

Dr. Gasser: Is there any objection to the classical view that the immediate source of the potential is a concentration cell?

Dr. Blinks: It is probably due largely to the concentration gradient of KCl in *Nitella.* In one species of *Halicystis* there are practically no gradients across the protoplasm, so it must be due to some gradient within the protoplasm itself. Finally in *Valonia* we have the potential in the wrong direction for the large KCl gradient, which shows that there must be some still larger potential directed inwardly. The question remains, what is this gradient. I think we are beginning to get some evidence. Obviously there must be something continuously generated by the cell to supply the energy for the continued current. This is probably derived from the process of respiration, but it seems unlikely that the potential is actually due to an oxidation-reduction system.

Dr. Cole: It would look as though this current, produced in *Halicystis,* would, in a couple of days, discharge all of the ions of a 0.5 molar uni-univalent salt solution in the volume of the cell.

Dr. Blinks: That is probably the right order of time. I once made a calculation for the time to do this in *Valonia,* where the potential and current is about one-tenth as large, and it came out to be about one month. That was assuming a sharp moving boundary, which, of course, would not occur.

Dr. Fricke: The amount of electrical energy which is released from *Halicystis,* according to the data given by Dr. Blinks, is so large that a comparison with the energy which may be expected to be available due to metabolism would appear to be of interest. A potential of 70 millivolts and a current of 10 microamperes correspond to $\frac{1}{2} \cdot 10^{-3}$ gr. cal./hour. As a figure for the metabolistic rate we may use 1 gr. cal./hour, gr., and for the active mass of *Halicystis* 10^{-3} gr. This gives 10^{-3} gr. cal./hour, which is of the same order as the electrically released energy.

Dr. Cole: Has there been any attempt to measure the heat production in these cells when stimulated?

Dr. Blinks: No. It might be difficult on account of the relatively small volume of the living protoplasm in these large cells.

Dr. Cole: In this matter of discharging the cell continuously over a period of a relatively long time, since the potential remains so nearly constant there are comparatively few disturbing factors entering in. The passage of the current itself is not a disturbing factor in the sense that the hydrogen ion is?

Dr. Blinks: Not with such currents as these (5 to 10 microamperes). If we apply progressively higher voltages in the external circuit, so as to increase the current outward across the protoplasm, there is, at first, a small decrease of the measured potential, due to polarization, then finally a point is reached where the potential goes through a complete reversal. It recovers when the outward current is stopped. Inward currents, up to very large values, only serve to increase the potential by polarization—up to 80 or 90 millivolts positive.

Dr. Cohen: Is the reversal exactly the same effect as that produced by the internal increase of alkalinity?

Dr. Blinks: The effects are so much alike that I think the same mechanism is involved.

Dr. Mudd: The assumption of a continuous non-aqueous layer at the cell surface is clearly of advantage from the point of view of simplicity. Is this assumption necessitated by the experimental data also?

Dr. Blinks: Some other might do as well. It is easier to start with a simple substance. We do not know, of course, that the protoplasmic-surface is simple.

Dr. Mudd: Is there anything to exclude the possibility that the protoplasmic surface should contain more than one component?

Dr. Blinks: It might contain a mixture of substances, or an amphoteric substance, to account for the accumulation of both anions and cations.

Dr. Gasser: About the mosaic notion of the plasma membrane—why should the surface form in mosaics unless there are many little cells at the surface of the main cell?

Dr. Cohen: That would be all right if you make the assumption that the cell is a perfectly homogeneous system localized in the cell and, therefore, on the surface of the cell.

Dr. Mudd: There are several classes of surface-active substances in protoplasm. It is difficult for me to conceive of a mechanism by which one of these, exclusively, could form the protoplasmic surface. White blood cells are perhaps instructive in this connection. These have been described by Kite, I believe correctly, as naked protoplasm. These cells may be observed, when studied with the cardioid dark-field condenser, to form long thread-like or veil-like processes. The formation of such processes may involve large spontaneous increase of surface. Such spontaneous increase of surface, in the case of the myelin

forms of lecithin, has been attributed, by Leathes, to the peculiarities of the lecithin molecule. These processes on white cells resemble the myelin forms of lecithin, and Fauré-Fremiet has found considerable data to indicate that they are, in fact, due to the presence of lecithin in the surface. The wetting and spreading properties of white blood cells, on the other hand, we have found suggestive of protein in their surfaces. At all events, it is difficult to conceive of so mobile a structure, which forms the limiting surface of the complex system protoplasm, and which undergoes rapid alterations of slope and surface area, and which spreads over and includes foreign particles, as composed of one homogeneous substance.

Dr. Osterhout: The mosaic theory of the cell surface seems highly improbable, because a mosaic can exist only in a solid, and the protoplasmic surface in these plant cells always acts like a liquid *i. e.* it rounds up in contact with water, and in every way shows true surface tension.

Dr. Blinks: No doubt all surface active substances of the protoplasm would tend to migrate toward the surface and become concentrated there. But they would stay in the aqueous phase; they would not produce a *new phase* on that surface unless altered in some way, as by denaturation of a protein, esterification of a soap, etc. Such an alteration might occur more readily with one of the surface active substances, than with the others, giving a single substance in the *non aqueous* phase.

Dr. Gasser: Is the surface a constant thing at all? May it not be just a statistical condition dependent on the molecular species available at the moment for concentration in the surface?

Dr. Blinks: Quite possibly. There might even be a definite alteration of properties in time, as suggested by G. E. Briggs (Cambridge University,) with cationic permeable periods alternating with anionic permeable ones. The effects of pH changes on the inner surface of protoplasm convince me that it may be extremely sensitive to metabolic changes.

Dr. Abramson: The cell is fully permeable to water, is it not? Considering that fact, it is not necessary to go far to prove that the cell membrane is heterogeneous. If water goes in and out of the cell, water molecules must go in and out of the oil film, so that the oil film will always have some water areas, over a time average.

Dr. Blinks: Dr. Shedlovsky's measurements on the mobility of potassium and sodium ions in guaiacol were done with the latter nearly saturated with water, were they not?

Dr. Shedlovsky: The water concentration in guaiacol amounted to about 4%.

Dr. Cole: A membrane a fraction of a micron thick might allow considerable water transport.

Dr. Gasser: I do not think that heterogeneity means the same as mosaic. The cell surface must be heterogeneous, but it was the mosiac structure I was discussing.

Dr. Abramson: Please define what you meant by a mosaic. Is each mosaic combined with each neighboring mosaic with something like a chemical bond, or is a Gibbs excess concentration considered a mosaic?

Dr. Gasser: By mosaic I meant discrete areas of permeability, some having one nature, some another, the areas being arranged like the tiles in the floor. In this form the mosaic theory appeals to me as being a very artificial one. One thing that struck me is that, after all, the velocity of propagation of the action potential in *Nitella* is not very different from what it is in some nerves: 2 cm. per sec. for *Nitella* which may be compared with 30 cm. per sec. in the slowest frog nerves. Another thing, do you attach any importance to the fact that the action-potential has an initial spike-like form followed by a more prolonged portion rising to a second crest. The second part has the same relation to the spike as does the after-potential in nerve, but it is relatively very much larger. Do you think that the two crests may mean two processes occasioned by the presence of two surfaces?

Dr. Blinks: Probably both are involved. For example, when KCl is applied to *Nitella* there is first a mobility effect on the outer surface, and then a secondary effect, possibly due to the entrance and combination of KOH with some acid of the cell, increasing the pH at the inner surface. I should like to raise the question as to the possibility of such a high mobility of potassium ions in a non-aqueous phase as is postulated by Osterhout for *Nitella*—a mobility nearly 75 times that of chloride. Theoretically there may be grounds for doubting this. Do Dr. Shedlovsky's measurements on guaiacol bear upon this?

Dr. Shedlovsky: I would like to mention one thing rather than the interpretation you asked for. It was very interesting to observe in working on the conductivity of alkali guaiacolates in guaiacol that the conductivity of a certain concentration of potassium guaiacolate in anhydrous guaiacol was very much different from that of the same concentration in guaiacol saturated in water. The conductance was 30 times greater in the water-saturated solvent than in the dry solvent. On the basis of dielectric constant alone you would not expect any such change in ionic mo-

bilities, and the explanation for this tremendous change in conductance is probably due to the difference in the size of ions rather than in the dielectric constant as such. The experiments of Kraus and Fuoss showed a similar tendency. In three expressions they derive, relating to the dissociation constant with the dielectric constant of the medium, and with the distance of closest ionic approach, the distance of closest approach is, at times, much more important than the dielectric constant. This is interesting in connection with speculations on the source of potentials arising from diffusions. It is possible that, in the living cell, there may be changes in the water content in the non-aqueous phase, running parallel with different salt concentrations, so that, I think, one should be very careful in making the assumption that the mobility of the ions remains constant—it may or may not.

Dr. Müller: Is the change of mobility due to change of hydration?

Dr. Shedlovsky: One must be careful to distinguish between the mobility of ion as ion and the mobility of the ion as constituent. The significant thing is the mobility of the ion constituent, and that involves the mobility of the ion itself and the degree of dissociation. The mobility of the ion itself may not be changed very much, but the mobility of the ion constituent may be changed tremendously, if the degree of dissociation changes. Another thing in media of really low dielectric constant, around 8 or 10, the conductivity curves frequently show a rather interesting minimum, with increased concentration. Dr. Fuoss accounts for that on the assumption that there exists a possible equilibrium between the ions and undissociated molecules. By simply locating the concentration corresponding to the minimum, and using these assumptions, he is able to reproduce the experimental curve from the theoretical curve, or vice versa.

Dr. Blinks: You would say that the effects of the added water would be upon both anion and cation, increasing the mobilities of both and not exaggerating the mobility of one?

Dr. Shedlovsky: The greater effect would be in the increased mobility of the ion constituents, by increasing the degree of dissociation. If we have any evidence that would point to a constant water content of the non-aqueous phase, I think that none of this material ought to be worried about.

Dr. Blinks: I think there is not any evidence, but it seems probable that all parts of the cell are pretty well saturated with water.

Dr. Shedlovsky: There may be an appreciable salting in, or out, of water.

THE MEANING AND CALIBRATION OF THE pH SCALE

D. A. MacInnes

It is not necessary to emphasize the importance of the subject of this discussion. There is no biological or chemical journal that does not make many references to hydrogen ion concentration, hydrogen ion activity and to pH values. This is, of course, natural since hydrogen and hydrogen ion are important constituents of water, and water constitutes a large part of ourselves and of our environment. Furthermore, the unhydrated hydrogen ion is the proton which is, at present reckoning, one of the three or four elementary particles of which our universe is constructed. The fundamental interest of the subject has also been shown by the investigations of Bronstead in Copenhagen and Conant and Norris in this country. They have extended the ideas of acid and base from water to other solvents. According to these workers the relation between the generalized acid A and the generalized base B is

$$A = B + [+]$$

in which $[+]$ is the proton. In this form the relation between an acid and a base closely resembles that between that of a reductant Re and an oxidant Ox in an oxidation reduction reaction. Such a reaction may be written

$$Re = Ox + [-]$$

in which $[-]$ is the electron. Thus one of these fundamental types of reaction involves the proton and the other the electron. There is, however, not enough time to discuss the subject in its more generalized relations and we will confine our attention to water solutions.

The gradations from acid on one hand through neutral to basic are now, almost universally, stated in terms of Sorensen's pH scale. In actual use this pH scale is established as follows. Galvanic cells, which may be schematically represented by the typical cell:

$$H_2,\ \text{solution A},\ \text{saturated KCl},\ 0.1\ N\ \text{KCl},\ \text{HgCl},\ \text{Hg} \qquad (A)$$

are set up and their potentials, which we will denote by E, are measured. The details of such a cell are as follows. The hydrogen electrode, which consists of a sheet of platinized platinum over which hydrogen gas is flowing, is inserted into "solution A," the pH of which is desired. This solution makes a liquid junction with saturated potassium chloride, and this in turn makes another liquid junction with a reference calomel half cell. This half cell consists of a mercury electrode which is in contact with a potassium chloride solution (frequently tenth normal) which is saturated with mercurous chloride. Now the pH value is connected with the electromotive force E of this cell by the simple relation

$$pH = \frac{E - E_0}{RT/F} \qquad (1)$$

in which R, T and F are the gas constant, the absolute temperature, and the faraday respectively, and E_0 is a constant which I will have occasion to discuss fully later on. Physically it is the electromotive force of the cell when it contains a solution which has a pH value of zero, which is approximately that of normal hydrochloric acid.

Such measurements as we have just described are either inconvenient or impossible in many cases, particularly with solutions of interest in biological work, and a number of alternative methods have been developed for determining pH values. The most useful of these are the colorimetric or indicator method, the quinhydrone method, and the glass electrode method. However, these methods are considered trustworthy only in so far as they agree with the hydrogen electrode method briefly outlined above. The pitfalls encountered when an attempt is made to do accurate work with indicators are many. As you know there are "salt errors" and "protein errors" and each indicator is a problem in itself. However, I must mention with admiration the work of Hastings and Sendroy as a beginning in the clearing up of this difficult field. The quinhydrone method has many of the difficulties of the hydrogen electrode and has a limited range of applicability. The glass electrode is admirably suited to work with the solutions encountered in biological research. In our most recent forms the fragility inherent in the earlier types has been eliminated. It is, however, necessary to compare such electrodes with the standard hydrogen electrode. With electrodes of the best glass for the purpose at present available, such a comparison shows that the glass electrode is accurate within the pH range 1 to 8. At pH = 11, the error is about one tenth pH unit.

If we regarded the pH simply as a variable that must be kept constant during a given set of experiments, it would only be necessary to agree upon an arbitrary value of E_0 in equation 1. However, pH values are fortunately much more useful than this. With them it is often possible to understand chemical reactions in solutions such as, for instance, the reaction between car-

bon dioxide and other components of blood or plant sap. However, for this purpose it is necessary to study the relation between the pH values and the hydrogen ion concentration, $C_H{}^+$, or hydrogen ion activity, $a_H{}^+$. Two relations frequently given are

$$pH = -\log C_H{}^+ \qquad (2)$$

and

$$pH \equiv -\log a_H{}^+ \qquad (3)$$

but the unforunate fact is that the first of these equations is not true and the second cannot be proved thermodynamically, for reasons to be outlined below. If equation 2 were valid it would be possible to establish our pH scale by calibrating with solutions for which $C_H{}^+$ is known. According to Arrhenius' theory of dissociation, we could obtain the hydrogen ion concentration, of hydrochloric acid for instance, by means of the relation: $C_H{}^+ = C\,\Lambda/\Lambda_o$, in which C is the total concentration and Λ and Λ_o are the equivalent conductances of the acid at the concentration C and at infinite dilution respectively. However, from a practical point of view, we would get a different value of E_o if we used 0.1 N hydrochloric acid than if we used say 0.001 N, for the calibration. Also we now know that the assumptions upon which this computation is based are erroneous, though a discussion of the matter would take us too far afield at this point. This brings us to a discussion of equation 3.

The activity is, as you know, a conception originated by Professor G. N. Lewis. In the present case the activity of the hydrogen ion constituent $a_H{}^+$ would be *defined* by the relation

$$E - E_o = \frac{RT}{F} \log a_H{}^+ \qquad (4)$$

if the potential at the hydrogen electrode were the only one that changed when the concentration of "solution A" is varied. E is once more the potential of a cell of the type represented by (A). The potential can, however, also change at the liquid junction between this solution and the saturated potassium chloride. By choosing saturated potassium chloride as one of the solutions, this effect is probably minimized, but, as we shall see, there is no way of telling, with certainty, whether such a technique is successful or not.

To state the difficulty in other words, there is no way of deciding what portion of the measured potential of a cell is located at any particular point. One school of thought, which includes particularly physicists, locates all the potential outside the cell and places it at the points of contact of dissimilar metals.

If, however, it were possible to obtain values of the activities of ion constitutents, such as that of the hydrogen ion $a_H{}^+$, it would, theoretically at least, be possible to compute values of the liquid junction, since its value, E_j, can be found from the relation

$$E_j = RT/F \int t_i d \log a_i \qquad (5)$$

in which t_i is the transference number of ion species i, and a_i is the corresponding ion activity. This evidently requires that we know all the individual ion activities and the transference numbers of all the ions in the solutions in contact at the liquid junction. The value of E_j unfortunately also depends not only upon the solutions in contact, but upon the way they are mixed. Furthermore the computation of the potentials of these liquid junctions taxed the ingenuity of such masters of mathematical physics as Max Planck, even after he had made simplifying assumptions. We are thus in the position that we cannot obtain hydrogen ion activities without correcting for the liquid junction, and we cannot correct for the liquid junction without hydrogen ion activities. Guggenheim, in a series of stimulating papers, has gone so far as to say that single ion activities have no meaning, largely because of this dilemma. It is also true that the ion activity depends not only upon the ion constituent itself, but also upon its environment, from which, of course, it cannot be separated.

Although single ion activities cannot be measured it is possible to determine "mean ion activities" by a number of thermodynamic methods involving no special assumptions. Two of these methods are the freezing point determinations and the measurement of potentials of concentration cells without liquid junctions. Though it is not possible here to demonstrate this, I will illustrate the ideas involved with a weak acid (say acetic acid) as an example. According to the Ostwald dilution law we have,

$$K' = \frac{[H^+]\ [Ac^-]}{[HAc]} \qquad (6)$$

in which the brackets represent the concentrations of the substances enclosed and K′ is the ionization or mass law constant. We now know that this is true only at infinite dilution and that the correct thermodynamic expression is

$$K = \frac{(H^+)\ (Ac^-)}{(HAc)} \qquad (7)$$

in which K is the thermodynamic ionization constant, and the parentheses represent the activities

of the substances enclosed. This can be put into the form:

$$K = \frac{[H^+]\quad[Ac^-]}{[HAc]} \quad \frac{\gamma_{H+}\ \gamma_{Ac}}{\gamma_{HAc}} \qquad (8)$$

in which the γ values are the activity coefficients which convert concentrations into activities. Now although we are not able to assign definite values to the individual activity coefficients it is possible to tell, within limits, what values they approach for very dilute solutions. The activity coefficient for the undissociated acid γ_{HAc} is very nearly unity and will not be considered in what follows. The product of $\gamma_{H+}\ \gamma_{Ac-}$ will be replaced by γ^2 which is the square of the mean ion activity coefficient.

Professor Edwin J. Cohn[1] has made the suggestion that if an accurate value of the thermodynamic ionization constant of a weak acid could be obtained, it could be used as a basis for the calibration of the pH scale in terms of activities. Shortly before that, MacInnes[2] and Sherrill and Noyes[3] had shown how the thermodynamic ionization constants could be found by the use of conductance measurements with the help of the Debye-Hückel theory. MacInnes and Shedlovsky[4] have recently obtained the necessary conductance data on acetic acid for the precise computation. In outline the computation is as follows. Values of K′ as defined by equation 6 are first computed by a method in which a necessary correction is made for "interionic attractions." These are related to the thermodynamic constant by the relation.

$$K = K'\,\gamma^2 \qquad (9)$$

Now it is known from the Debye-Hückel theory that for low ion concentrations at 25°

$$-\log\gamma = 0.506\sqrt{C_i} \qquad (10)$$

in which C_i is the ion concentration. These two relations can be put into the form:

$$\log K = \log K' - 2 \times 0.506\sqrt{C_i} \qquad (11)$$

Thus, if that theory is valid, for low ion concentrations a plot of values of log K′ against $\sqrt{C_i}$ should be a straight line with an intercept at log K. This has been found to be true and the method yields a value of 1.754×10^{-5} for the thermodynamic ionization constant of acetic acid. Recently Harned and Ehlers[5] by an entirely different method, involving concentration cells without liquid junctions, obtained the value -1.754×10^{-5} This agreement is most gratifying and makes it probable that we have here an accurate value of a constant of nature. A similar investigation for the considerably stronger acid, chloro-acetic, also yielded results in accord with the Debye-Hückel theory for the dilute solutions.

It now remains to calibrate the pH scale with the aid of these thermodynamic constants. Assuming equation 3, the equation

$$K = \frac{(H^+)\ (Ac^-)}{(HAc)} \qquad (7)$$

in which the parentheses represent the activities of the substances enclosed can be rewritten in the form

$$pK = pH - \log\frac{[Ac^-]}{[HAc]} - \log\gamma \quad (12)$$

in which pK is the negative logarithm of the thermodynamic ionization constant. Now if we measure the pH values of a series of buffer solutions in which the concentrations $[Ac^-]$ and $[HAc]$ are equal then, since γ approaches unity and log γ approaches zero for very dilute solutions, the pH value should approach pK *if we have chosen the correct scale for the pH values.* (A small correction for the shift of the ionic equilibrium is necessary.) If the Debye-Hückel theory is valid we should plot the observed pH values against the square root of the concentration in order to make the extrapolation. The computation therefore consists in obtaining a series of values of pH corresponding to different values of E_o in equation 1 until we find a value which will yield pK accurately on extrapolation. This has been done for both acetic and chloro-acetic acid buffers, using data recently obtained in the laboratory of the Rockefeller Institute by Mr. Donald Belcher. The data for the former gives a value of 0.3358 for E_o at 25° and the latter, the closely agreeing value 0.3357. The usually accepted value is 0.3376. As explained earlier in this paper, this difference would not be important if we were interested only in reproducible pH values, but becomes of interest if we intend to relate the pH values to chemical equilibria in the solutions under study. It is of importance that the slope of the lines obtained by the method of plotting just mentioned is very nearly that demanded by the Debye-Hückel theory. There are, however, deviations from this simple theory, at the higher concentrations, which might indicate that the liquid junction potentials change somewhat with the concentration.

The question may be reasonably asked whether this procedure has any advantage over that of using hydrochloric acid for the standardization, since the ionization relations of that substance are presumably simpler than those of the weak acids. It is, however, an unfortunate fact that the cali-

bration with a strong acid gives a different value for E_0 and the accuracy obtainable is much lower than that attained with the buffers described above. This difference is, undoubtedly, due to the larger potential at the liquid junction when a strong acid is used, and the greater dependence of the value of this potential upon the way the junction is made. Furthermore, calibration with a strong acid means doing it at low pH values, where for biological and much other work the interesting field is roughly between the pH values 4 and 9.

Some recent measurements with carbonate-bicarbonate buffers at relatively high pH values cannot readily be explained by the current theories, and must receive further study.

The chief ideas in this paper may be roughly summarized as follows: The pH values of solutions are related by a simple formula to the potentials of certain galvanic cells containing the solutions. These pH values cannot be interpreted as measures of hydrogen ion concentrations. Furthermore, hydrogen ion activities cannot be defined thermodynamically, and may have no physical meaning. Mean ion activities are, however, possible of measurement by thermodynamic methods. By the proper choice of standards the pH scale can be adjusted so that the values in that scale will measure, as nearly as possible, "the mean ion activities of the hydrogen ion constituent."

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DISCUSSION

Dr. Abramson: Does the dielectric constant of the solvent come into the theoretical slope?

Dr. MacInnes: Yes. It enters as the inverse three halves power.

Dr. Abramson: We had similar success with the dielectric constant of the solvent in employing the general theory of electrophoresis.

Dr. Müller: Are the deviations from the Debye-Hückel equation connected with the fact that the hydrogen ion is small? The nature of the deviations which you observe is identical with what one would get by using the higher approximations of the theory.

Dr. MacInnes: The hydrogen ion does not seem to be small. The data on hydrochloric acid fit the Debye-Hückel theory in its simple form. It behaves as if it had a "distance of closest approach" of about six angstroms. If this distance gets below about three angstroms, corrections, for higher terms, such as you and Gronwall, La Mer and Sandved worked out, must be made.

Dr. Michaelis: There are, of course, no naked protons in solution.

Dr. MacInnes: Also the simplest compound of proton with water, that is H_3O^+, is still too small, but it is probably hydrated extensively enough to increase its size so that the extended theory I have just mentioned is unnecessary.

Dr. Fricke: The hydrogen ion is undoubtedly quite large. It may be well to remember that an explanation is necessary to account for the large velocity in view of the size, as we now understand it to be.

Dr. MacInnes: Some people think it is due to the proton jumping from one ion to another. I am not completely convinced of this.

Dr. Fricke: It is undoubtedly related to the fact that the hydrogen ion is a dissociation product of water.

Dr. MacInnes: That certainly has something to do with it.

Dr. Abramson: The constant K′ which you use in your equation for ion activity involves conductance measurement. You have a mobility related explicitly to an activity.

Dr. MacInnes: They are not related. We use conductance simply as an indicator of ion concentration and compute the activities by using the Debye-Hückel theory.

Dr. Abramson: It is not a method of getting activity coefficients?

Dr. MacInnes: No. My discussion simply shows that in a dilute solution, our use of conductance measurements gives the right concentrations if you accept the Debye-Hückel theory as valid for these dilute solutions.

Dr. Fricke: Can you experimentally distinguish between two ions kept together by electrostatic force and an actual undissociated molecule?

Dr. MacInnes: No. It is a much discussed question. Bjerrum ascribes departures from the simple Debye-Hückel theory to "ion associations" due to the action of intense electric fields when small ions get close together. Gronwall, La Mer and Sandved explained the same departures by extending the mathematics implicit in the simple

theory. Recent commentators regard the two treatments as having formal differences only.

Dr. Fricke: I presume that the formation of the real molecule would be distinguished by a definite change of energy.

Dr. MacInnes: I do not know that there is any clear way of distinguishing between them. It is a question that is very much discussed.

Dr. Cole: Another line of evidence on that has been published by Woodward, working in Leipzig on the Raman effect on strong electrolytes. He has found in strong electrolytes no evidence of undissociated molecules.

Dr. Mudd: Do amino-acids and proteins, at their isoelectric points, have the electric moments of doubly ionized particles?

Dr. MacInnes: Yes, you can raise the dielectric constant of water by adding certain amino-acids.

Dr. Michaelis: How much is the dielectric constant changed?

Dr. MacInnes: The work of Hedestrand shows that normal aqueous solutions of some amino acids have dielectric constants higher than water by nearly thirty percent.

END OF COLD SPRING HARBOR SECTION

THE IRRADIATION OF BIOLOGICAL SUSPENSIONS BY MONOCHROMATIC LIGHT (THE EFFECT OF ULTRA-VIOLET LIGHT ON A PLANT VIRUS AND BACTERIA)

DR. B. M. DUGGAR[1] AND DR. ALEXANDER HOLLAENDER[2]
Laboratory of Plant Physiology, University of Wisconsin

For the irradiation of certain biological suspensions with measured quantities of monochromatic light, special apparatus has been designed. Intense sources of monochromatic light have either been adapted or constructed. Special cells and stirrers have been built. The energy was measured by the usual thermopile-galvanometer arrangement. The apparatus was built around a B. and L. quartz monochromator put at our disposal by the manufacturer, through the Radiation Committee of the National Research Council.

The virus of typical tobacco mosaic, approximately purified, was exposed in V/100 at about 0° C. The virus suspension was made up after pasteurization in physiological salt solution. The bacteria used were *Serratia marcescens, Bacillus subtilis,* vegetative and spore stages, *Bacillus megatherium* spore form. For the determination, both of the lethal effects on bacteria and of inactivation of the virus, the two materials were combined in the same suspension, so that comparative values might be obtained. Exposed materials were accompanied by controls similarly treated, except as to protection from radiation.

After exposure, dilutions of the bacteria were made in physiological salt solution of the irradiated cultures and the unirradiated controls, and by plating these on agar media, a definite quantitative comparison was possible. The percentage inactivation of the virus was determined by inoculation of tobacco plants and by comparing the incidence of disease induced by the exposed virus as compared to that induced by unirradiated virus.

Inactivation of the virus is confined to wave lengths shorter than about $\lambda 3100$ angstrom units. The energy required to produce perceptible effects at approximately λ 3100 angstrom units is more than 100 times as much as is necessary at λ 2650 angstrom units. The energy values of incident light, representing 100 per cent. killing of the bacteria are far below the values having any measurable effect on the virus. For both of these biological materials, using the range from λ 2537 angstrom units to 3100 angstrom units, the greatest influence is at λ 2650 angstrom units. The resistance ratio of virus to bacteria as represented by these results is about 200:1.

Apparently there is very little relation between heat resistance and light resistance. B. subtilis (spore form) will survive extended boiling for fifteen minutes, the vegetative stage will scarcely withstand fifteen minutes at 65° C. The virus is inactivated with an exposure of ten minutes at 90° C.

There is only little difference in the energy necessary to kill the spore stage and the vegetative stage of B. subtilis; but the energy to inactivate the virus in the same suspension is about 200 times the energy necessary to inactivate both forms of B. subtilis.

In relation to wave length, bacteria and virus, as well as several other biological materials show the same sensitivity.

[1]Professor of Plant Physiology.

[2]National Research Fellow in Biological Science.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 1.)

REGIONAL DIFFERENCES IN THE ORGANIZATION CENTER OF THE AMPHIBIAN EMBRYO

DR. EDMUND K. HALL
Instructor in Anatomy, University of Louisville School of Medicine

Up to the present time, sufficient data have not yet been accumulated for a complete understanding of the inductive reaction which takes place between the organization center and the ectoderm of the gastrula during the normal development of the Amphibian embryo. Evidently, there are several possibilities as to the manner in which this reaction occurs: Each part of the archenteron roof may induce specifically in the superjacent ectoderm the specialized structures which later develop there. Or, on the other hand, the archenteron roof may give rise to a merely general neural stimulus; the character of the organ which arises from any particular region of the medullary plate would then depend on factors present in the ectoderm, or perhaps on the "organism as a whole."

As a matter of fact, Spemann has found that both possibilities are realized when inductions occur in the lateral and ventral ectoderm. "Head or-

ganizer" induces a brain in all parts of this ectoderm and so seems to exert a specific inductive stimulus for this organ. "Trunk organizer," on the other hand, induces a spinal cord when acting on the posterior ectoderm, and a brain when acting on the anterior ectoderm; its stimulus therefore seems to be a more general one than that of head organizer, and the type of organ formed seems to depend on the level of the embryo at which the graft is acting.

The results reported in last Tuesday's seminar deal with the action of head organizer and trunk organizer when they are acting, not upon the lateral and ventral ectoderm of the gastrula, as in Spemann's experiments, but upon the ectoderm of the future medullary plate. Trunk organizer, when it underlies the anterior end of the medullary plate, seems under these conditions to exert a specific stimulus, and not a general one, for the anterior end of the neural plate is much elongated, and is as narrow as it is posteriorly. In such animals, curious elongated heads develop, in which some of the head organs are either lacking, or very rudimentary. When head organizer underlies the posterior portion of the medullary plate, it seems to exert a merely general neural stimulus, and not a specific one, for the development of the spinal cord proceeds normally. In this case, of course, the level at which the graft is acting has in some way determined the type of reaction.

Spemann's results and those of this investigation may therefore be summarized by stating that both head organizer and trunk organizer may either act to induce specific structures, or may give rise to a merely general inductive stimulus, the kind of organ produced depending on the level at which the graft acts. The type of reaction which occurs depends, of course, on the experimental conditions.

(This article is based on a seminar report presented at the Marine Biological Laboratory on August 8).

BOOK REVIEW

The Freshwater Algae of the United States, Smith, Gilbert Morgan, XI—716 pp., 449 fig. McGraw-Hill Book Co., Inc. New York, N. Y. 1933.

The appearance of Professor Smith's textbook places American students at a great advantage in the study of freshwater algae. Dependent as they have been upon foreign texts for introductory information, it is not surprising that many have written upon American algae with an inadequate general knowledge of the group. The British books, which have been our most available sources of instructional material, have become somewhat obsolete through the rapid increase in life-history studies, with their consequent revisions in classification. The book under consideration gives a most needed and acceptable resumé of current research. In general, after preliminary chapters on the nature and evolution of algae, their distribution and ecology, methods of collection and study, the eight major groups recognized are discussed in chapters each prefaced by a general account of the structure, types of reproduction, life histories and evolutionary trends of the group. The book is clearly a textbook of morphology, life history, ecology and general systematics as they concern the algae. It is not intended to serve as a taxonomic manual, although much taxonomic information is included, and through literature citations the student is directed to suitable detailed sources. The major groups and genera are described and figured, but no attempt is made to list all the known species in the larger genera. In small genera all the American species are mentioned, with their more obvious distinctions. The system of classification adopted is conservative, particularly in the Myxophyceae, which will aid its use by non-specialists. The illustrations, designed to show not only the morphology of each genus, but often the reproduction as well, are very clear and well executed. With some exceptions, most frequent among the flagellate genera, they are original.—W. R. Taylor.

Professor and Mrs. Charles D. Snyder with their son Thoma are planning to spend a couple of weeks in the White Mountains. At the end of that time they plan to come to Woods Hole for a visit before they return to Baltimore. Their daughter, Francina, is a guest of Professor and Mrs. Jennings in the Gansett Woods.

Five of this year's best sellers have been ordered for the club, Hervey Allen's "Anthony Adverse", Gladys Carroll's "As the Earth Turns", Robert Herrick's "Sometime", the two volumes of Theodore Dreiser's "Gallery for Women", and Pearl Buck's latest novel, "The First Wife".

Last December, Dr. E. C. Cole was one of the ten of the faculty of Williams College to receive an annual grant for research of $400.00 from the "Williams 1900 Fund."

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Jean M. Clark, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

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PAST AND FUTURE

It gives us pleasure to announce that one hundred and sixty-six dollars and five cents have been turned over to THE COLLECTING NET Scholarship Fund by the Penzance Players which represents the proceeds from their play "You Never Can Tell," so ably directed by Mrs. George A. Baitsell.

On Monday evening, August 28 THE COLLECTING NET will present a combination lecture and motion picture on "Whaling Lore" by the son of an old New England whaling captain in the auditorium of the Marine Biological Laboratory.

We sincerely hope that the community will generously support this program, not only for the money that it will bring to the scholarship fund but because "Whaling Lore" deserves it on the basis of its interest and educational value. It has been much improved orally and pictorially since Dr. Conklin wrote of it:

"Audience one of the largest ever assembled in our new auditorium—and safe to say as critical as you will ever address—lecture both interesting and profitable. I have heard only words of praise for your presentation of the subject—rare advantages of first hand knowledge."

THE VALUE OF SCHOLARSHIPS TO THE STUDENTS AT THE MARINE BIOLOGICAL LABORATORY

Dr. R. W. Gerard, Associate Professor of Physiology, University of Chicago

Having been asked to comment as to the value of the scholarships given worthy students each year for superior work in the Marine Biological Laboratory courses, and sponsored by THE COLLECTING NET, I take it that the question of the worth of scholarships in general need not be raised. Certainly, since the days when science changed from a pursuit of the wealthy amateur, as in the early days of the Royal Society of England, to the serious purpose of a corps of devotees of all resources and antecedents, it has clearly been impossible for the bulk of investigators to depend upon their own resources. Apparatus has become ever more complicated and expensive, techniques more intricate and training very prolonged. To meet these growing requirements and in tacit recognition of the contribution made by research to society as a whole, endowments have been steadily increasing in the form of university reserves, special research budgets, and particularly, fellowships and scholarships to aid the student at all levels of development, from the time superior ability becomes evident until it has been fully nurtured and productive.

The situation at Woods Hole, as the specific instance of these general conditions, is very interesting. Here, at one of the largest biological institutions in the world, at which gathers every summer an extraordinary variety and richness of scientific talent, there are given courses for the advanced training of some one hundred students a year. Speaking particularly for the physiology course, with which I am naturally best acquainted, the students are almost invariably college graduates, often with years of additional training, and have come here largely at their own expense and initiative to extend the training they have been able to obtain at the usual college and graduate school. A surprisingly large proportion of these students, probably well over half, have continued productive research in later years and, in fact, a very large number of the regular investigators who work here each summer have been recruited from the courses in years past. The students here constitute then, a selected group of embryonic investigators assembled from the entire country. Most of them, of course, need money badly; many eke out the summer's expenses by waiting table at the Mess and the like, but such tasks do not mix well with the requirements of irregular research hours. All possible financial aid is welcomed by the students, and, in fact, the number who have been able to return to Woods Hole in subsequent years and complete unfinished projects with the aid of COLLECTING NET scholarships must be very gratifying indeed to those who have contributed to their success.

I am happy, therefore, to lend the assurance of our experience in urging that these scholarships be generously supported.

Dr. George deRenyi has arrived for his summer's work at the Laboratory. He spent the early part of the season at the Bermuda Biological Station.

Dr. E. C. Cole, assistant and associate professor of biology for nine years at Williams College has been advanced to the rank of full professor.

ITEMS OF INTEREST

At its Annual Meeting, held on the tenth of August, the Woods Hole Oceanographic Institution re-elected its officers as well as five of the six trustees whose terms expired this summer. Lieutenant Commander E. H. Smith of the U. S. Coast Guard was elected to the Board to replace R. P. Scripps of Ridgefield, Connecticut, and the other members re-elected to serve until 1937 are: Newcomb Carlton of New York; Dr. T. H. Morgan of the California Institute of Technology; R. S. Patton of the U. S. Coast and Geodetic Survey; B. W. St. Clair of West Lynn; and the Hydrographer of the U. S. Navy Department.

The re-elected officers are Lawrason Riggs, Jr., Treasurer, and Henry B. Bigelow, Clerk.

Mr. C. H. Bostian and Mr. B. R. Speicher, graduate students at the University of Pittsburgh, are receiving the degree of Ph. D. this summer. They finished their theses during July, and took the final oral examinations in Woods Hole. The examining committees consisted of Dr. P. W. Whiting and E. A. Wolf of the Department of Zoology, Dr. John Donaldson of the Medical School of the University of Pittsburgh, Dr. Anna R. Whiting of Pennsylvania College for Women, and Dr. O. E. Nelson of the University of Pennsylvania. Mr. Bostian, who is assistant professor of zoology at the North Carolina State College of Agriculture and Engineering, had as his thesis subject, "Biparental males and biparental ratios in Habrobracon." Mr. Speicher's subject was "A morphological study of the effective period of 'Eyeless' and 'Glass' in Habrobracon."

Dr. H. B. Steinbach, instructor in physiology at the University of Pennsylvania, who received his degree of Doctor of Philosophy this June, has been teaching physiology course in summer school. After a visit at his home in Michigan he will work at Woods Hole in September and in Chicago during the winter with Dr. Ralph Lillie under a National Research Fellowship.

Dr. Maynard M. Metcalf, research associate in zoology at Johns Hopkins University, has come to Woods Hole with his family to spend several weeks at his cottage on Crow Hill.

Dr. C. C. Speidel left Woods Hole on August 10 for England, where he will participate in the International Congress for Experimental Cytology which opens on August 21. Dr. Robert Chambers will leave on August 14 to attend the congress.

Dr. Balduin Lucké left in the early part of last week for a vacation in Wyoming, where his wife has been spending the summer.

Dr. Oscar Schotté is leaving Woods Hole very shortly for New Haven, where he holds a research fellowship at Yale University.

Dr. Caswell Grave recently arrived at Woods Hole from the Tortugas Islands, where he, with the assistance of Mr. Paul Nicoll, has been studying the tunicates of the coral islands.

Dr. Jenning's son, Burridge, has come to Woods Hole for a part of his vacation. He has recently been taking a summer course at the University of Michigan.

Last week-end four Woods Hole investigators, Dr. Norma Furtos, Miss Arliner Young, Mr. Donald Costello, and Mr. Daniel Mazia, drove to Philadelphia. There Mr. Costello deserted the party and joined Dr. H. B. Steinbach, who was leaving for Detroit.

Dr. William Young, of Brown University, took a short leave of absence from Woods Hole in order to climb Mount Washington.

Mr. McInnes, of the Supply Department, took a baseball team composed of town people, collecting crew, and investigators to Vineyard Haven in the Nereis on Sunday afternoon, and brought back a 5-4 victory.

Dr. Joseph Hale, now an instructor in chemistry at Earlham College in Richmond, Indiana, returned to Woods Hole for the latter part of last week. He expects to spend a few weeks here later this month.

Dr. Edgar "Tony" Hill is leaving Woods Hole on Wednesday to return to his home in Kentucky, where he will spend a short vacation before returning to the Rockefeller Institute in the Fall.

Dr. Adam Böving, senior entomologist, Bureau of Entomology at the United States Department of Agriculture recently arrived in Woods Hole. He is an authority on the larval stages of beetles and is spending much of his time here with his old friend, Dr. August Krogh.

The Annual Meeting of the Trustees and Corporation of the Marine Biological Laboratory

The annual meeting of the Corporation of the Marine Biological Laboratory and of its Trustees convened on August 8. The Trustees' meeting, as usual, was divided into a morning and afternoon session. The question of finances, the relation of the Laboratory to the National Industrial Recovery Act, and the appointment of the Committee of Review were among the more important things discussed.

The members of the Corporation met at 11:30 on Tuesday morning. The Clerk read the minutes of the last meeting; and this was followed by a brief Treasurer's report by Mr. Riggs. The major part of his report is available in the Annual Report of the Laboratory published recently in the *Biological Bulletin*. Mr. Riggs emphasized the fact that the financial situation did not appear good in March, but that the gloominess prevalent then was not entirely warranted, and that the general financial condition of the Laboratory was better than that of many educational institutions.

In the report of the Librarian, Mrs. Montgomery formally announced the gift of the scientific library of the late Professor William Patten by his son, Dr. Bradley M. Patten. She spoke at some length of the high cost of foreign scientific magazines, especially those in Germany, and was in favor of having people here protest against the excessive charges now being made by certain scientific publications in Germany. She mentioned that one or two German publishers had already come to realize that it was necessary to keep down the cost of these publications, and had taken steps to do so.

In the Director's report, Dr. Jacobs spoke especially of the attendance and research productivity of the investigators at the Laboratory. He gave a series of figures showing that at the beginning of the season the attendance at the Laboratory was below that of the previous summer; but later figures showed that as early as the end of June the daily attendance at the Laboratory was actually greater than in 1932. The figure for August 7 of this year, compared to that for the same date last year, showed an increase of fourteen in the number of people working at the Laboratory. On Monday, August 14, 265 investigators had registered during the summer, as against 251 for the same date last year. Dr. Jacobs emphasized the fact that the number of investigators present this summer comfortably uses all of the facilities of the Laboratory without unduly taxing any one of them, and that these conditions were optimum for comfort and research productivity.

Dr. Jacobs exhibited three piles of reprints as indication of the research work accomplished during the past three years. The pile for 1932 was smaller than that for the previous two years, but he took pains to point out the fact that bulk is a poor measure of value and that the importance of the research work described in the publications for 1932 might be greater than in either of the two other years.

He announced the retirement on a pension of the following three workers at the Laboratory: John J. Veeder, who first began work here in 1899; George M. Gray, who began in 1891, and Ellis M. Lewis, who began in 1897.

The Director's report was followed by the election of officers of the Corporation. The Treasurer, Lawrason Riggs, and the Clerk, Dr. Charles Packard, were re-elected to their offices. Dr. W. R. Amberson and Dr. C. C. Speidel were elected to replace Dr. H. C. Bradley and Dr. C. E. McClung. The other six members of the group of eight whose terms automatically expired this year were re-elected to serve another four year term. They were: Drs. H. B. Goodrich, Wesleyan University; I. F. Lewis, University of Virginia; R. S. Lillie, The University of Chicago; T. H. Morgan, California Institute of Technology; A. C. Redfield, Harvard University; D. H. Tennent, Bryn Mawr College.

Under the head of new business, the only matter which came up was the giving of a formal vote of thanks to Dr. Patten for the gift of his father's library.

The meeting adjourned at 12:20 A. M.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the hole turns to run from Buzzards Bay to Vineyard Sound:

	A. M.	P. M.
August 16	1:21	1:26
August 17	2:15	2:21
August 18	3:02	3:08
August 19	3:46	3:53
August 20	4:26	4:37
August 21	5:05	5:18
August 22	5:43	5:58

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

THE COLLECTING NET

Vol. VIII. No. 8 — SATURDAY, AUGUST 19, 1933 — Annual Subscription, $2.00 Single Copies, 25 Cents.

COLOR CHANGES IN THE DOGFISH

Dr. G. H. Parker and
Helen Porter
Department of Zoology, Harvard University

Lundstrom and Bard have shown that the common dogfish *Mustelis canis* can change from light to dark according to the background. In experiments on the pituitary gland they have further shown that dogfishes deprived of this organ become light. Moreover, when pituitary extracts are injected into these light animals, they turn temporarily dark. The conclusion arrived at by these authors is to the effect that pituitary secretions control the color changes in this case, the animal being light when it is deficient in these secretions, and dark when it has an abundance of them.

In our experiments we have found that wherever nerves are severed, light patches or streaks appear on the skin of the dogfish. If cuts are made in dark fish, these light marks tend to disappear in a few days; if they are made in a light fish, they appear to be indefinitely retained. The marks (*Continued on Page 254*)

M. B. L. Calendar

TUESDAY, AUGUST 22, 8:00 P M.

Seminar: R. Rugh: "Heterochromatic radiation and early amphibian development."

R. E. Zirkle: "A non-linear relation between biological effect and ionizing power of alpha rays."

Leon C. Chesley: "Effects of X-rays upon cell oxidations."

P. S. Henshaw and D. S. Francis: "A response of Arbacia eggs to X-rays."

THURSDAY, AUG. 24, 8:00 P. M.

Dr. Robert Chambers and Mr. C. G. Grand, New York University: "Tissue culture technique and various aspects of the growth of normal and cancerous tissues."

FRIDAY, AUGUST 25, 8:00 P. M.

Lecture: Edwin Grant Conklin: "Science and Progress."

EVIDENCE OF THE PROTEIN NATURE OF PEPSIN AND TRYPSIN

Dr. John H. Northrop
Member, The Rockefeller Institute for Medical Research

While the behavior of enzymes has been systematically worked out in the last 40 or 50 years, very little advance has been made in the knowledge of their chemical nature so that it has frequently been assumed that they represent an unknown class of compounds. Indirect evidence has been obtained, however, that some, at any rate, are proteins. The rate at which they are destroyed by heat, for instance, is characteristic for the effect of temperature on proteins. The fact that they are adsorbed on finely divided particles is also a property of proteins more than of many other classes of compounds. Pepsin, in particular, seems to have protein-like characteristics, and in fact Peckelharing isolated an amorphous protein from gastric juice which was highly active and which he considered to be pepsin itself. He was unable, however, to show that the material was a

TABLE OF CONTENTS

THE MARINE BIOLOGICAL LABORATORY

pure substance, and the view that this protein was really the enzyme was never accepted. The writer has repeated Pekelharing's experiments several times in the last 15 years, but until recently had never been able to carry the purification any further. In the meantime Sumner reported the isolation of a crystalline protein from beans which appears to be the enzyme urease.

Nearly all attempts to isolate enzymes have been done with relatively small quantities of material and in rather dilute solution. Absorption methods have also been extensively used. If enzymes really are proteins, these are not favorable conditions for their isolation, since proteins are extremely unstable in dilute solution and are easily injured by adsorption on surfaces. The attempt to isolate pepsin was again undertaken three years ago from the point of view of protein chemistry, using only those conditions under which proteins are relatively stable, i.e., concentrated solutions and low temperature. The method was based originally on that of Pekelharing. The last step in Pekelharing's preparation consisted in dialyzing a protein fraction from gastric juice against dilute acid. Under these conditions a white precipitate is formed which is a protein and which contains most of the activity. This protein sometimes appears in a somewhat granular form and under the microscope looks as though it might be trying to crystallize. Many attempts were made to crystallize the protein without success. It was noticed finally that this precipitate dissolved if the suspension were warmed to 37° C. and reappeared again upon cooling. These are good conditions for the formation of crystals, and the experiment was repeated under varying conditions and especially with more concentrated solutions, since crystallization in general occurs more readily from concentrated than dilute solutions. A more concentrated suspension than usual was warmed to 37° C., and this solution was allowed to cool slowly to room temperature in a beaker. The next morning it was found to contain several grams of beautifully formed crystals in the form of double, six-sided pyramids. They were tested for activity and found to be highly active and also to be protein.

The activity is about 5 times that of the most highly active commercial preparation and the quantity of protein which can be transformed by the enzyme is quite extraordinary. An ounce of the crystalline pepsin under favorable conditions would digest about 1½ tons of boiled egg in 2 hours, or would clot about 600,000 gallons of milk, while it would liquefy about 10,000 gallons of gelatin in the same time. To imitate these reactions by chemical means would require a great deal of work and violent methods, but the enzyme accomplishes it without any heat effects and, what is still more remarkable, without anything happening to itself. So far as can be determined it is present after it has done its work just as it was when the reaction was started.

The next question was whether or not this digestive power was really a property of the protein or whether it was due to the presence of more highly active molecules accompanying the protein. This question can be answered in two ways. If it can be shown that the material is a pure substance or, in other words, that it contains only one molecular species, then it follows that the protein-like properties and digestive properties must both be attributes of the same molecule. Unfortunately, it is not possible to furnish definite, positive proof of the purity of any substance. It can only be stated that so far it has been impossible to separate it into two or more substances, and this statement may be made with respect to pepsin. The composition, optical activity and digestive activity remain constant throughout 7 successive crystallizations, and this would usually be considered satisfactory proof of the purity of a substance.

As a result of these experiments it can be said that no indication was found that the material was a mixture by the usual tests. Owing to the fact that it is a protein, however, it is quite possible that the crystals are a solid solution of several related proteins. The relation between protein and activity may, however, be tested in another way by comparing the loss in activity with the destruction of the protein. If the activity were due to some other molecule associated with the protein it seems probable that conditions could be found which would decompose or change the protein molecule without affecting the activity, or *vice versa,* whereas if the activity were a property of the protein molecule itself it would be expected that anything which affected the protein molecule would also affect the activity. It was found that this protein was denatured, that is to say, changed into an insoluble form, in very dilute alkali. This is quite unusual for a protein. A careful study of this reaction was made, therefore, and it was found that the loss in activity was just proportional to the amount of soluble protein transformed into insoluble protein when various amounts of alkali were added.

If a solution of pepsin is allowed to stand in dilute acid at 30° C. to 50° C. the protein hydrolyzes slowly so that the quantity of protein in the solution becomes less and less. Under these conditions it was found again that the decrease in activity was just proportional to the decrease in the quantity of protein present. Finally, it was found that the denatured protein formed by the action of alkali could be changed back, at least to a small extent, to the soluble form by allowing it

to stand for some time after the alkali solution had been partially neutralized. The soluble protein recovered in this way has the same activity as the original protein. These experiments, therefore, show that when the protein is denatured the activity is lost and when the protein is hydrolyzed the activity is also lost, and, furthermore, that none of the products originating from the hydrolysis of the protein have any appreciable activity. They are very good evidence that the activity is really a property of the protein molecule.

It is known that proteins are denatured by ultra-violet rays as well as by beta and gamma rays from radium. These reactions offer two additional ways of modifying the protein. Such experiments have been carried out by exposing pepsin solutions to ultra-violet light or radium rays, and the change in activity compared to the loss of protein nitrogen. The loss in activity under these conditions is again proportional to the decrease in protein concentration.

Dr. Herriott has prepared a crystalline acetyl derivative of pepsin by the action of ketene. There are six amino groups originally in pepsin so that it is possible to add at least six acetyl groups. As acetylation proceeds the activity becomes progressively less until it is equal to about one-half the original. A crystalline acetyl compound having about five acetyl groups and probably one amino group may be isolated from this solution. If acetylation is carried further there is an additional drop in activity and an insoluble compound is formed. The results suggest that five of the amino groups of pepsin can be replaced without destroying the activity but that acetylation of all the amino groups results in a total loss of activity.

It has been known for a long time that insoluble proteins take up pepsin and trypsin very readily from solution, and it has been suggested by Waldschmidt-Leitz that this reaction consists in the removal of the active group from the pepsin protein. When the experiment was carried out, however, and the complex analyzed for pepsin, the amount of activity present in the foreign protein is found to be just equivalent to the amount of pepsin protein present in the complex. This is analagous to the general reaction between proteins and nucleic acid and is dependent upon the pH. Such complexes may contain 60 to 70 per cent. pepsin. They can be quite easily separated into pepsin and edestin. If the active edestin crystals are stirred in cold sulfuric acid at pH 1.0 the pepsin dissolves out and may be isolated from the solution. The edestin crystals remain insoluble and are now inactive. The pepsin may also be recovered by allowing the edestin pepsin complex to autolyze at 37°C. and pH 2.0. Under these conditions the edestin is digested and the pepsin may be recovered from the solution.

There seems reason to believe, then, that pepsin (and probably urease) are proteins; but evidently, since there are many hundreds of enzymes, it can not be concluded at once that all enzymes are proteins. There is some reason to believe that trypsin is also a protein, since it has been known since the time of Kühne to be associated with the protein fraction. In fact, it had been supposed by some workers to be a nuclear protein, but Levene was able to show that this was not the case.

An attempt was made to continue the methods used by the earlier workers and to isolate a crystalline protein from pancreatic extracts. The problem turned out to be a difficult one, and a great deal of work was done before any encouraging results in the way of either a crystalline product or a product of constant activity was obtained. The most hopeful method seemed to be a combination of fractionation with acid and salt, as was done in the case of pepsin, but with trypsin it was necessary to use ammonium sulphate. A protein fraction was eventually obtained which had a constant activity and gave some indication of crystallization. The work was made difficult by the very unstable nature of the protein. This unfortunate property made it impossible to allow a solution to stand for more than a few hours, so that the usual process of crystallization, which consists in allowing a solution to concentrate or cool very slowly, could not be used. After a large number of unsuccessful attempts, Dr. Kunitz was able to secure definite, regular crystals by the very cautious addition of strong ammonium sulphate to rather concentrated solutions of the protein. The crystals are rather small and are of the cubic system. The proof that this material is a pure substance is still more difficult than in the case of pepsin, since it is more unstable. A large number of solubility experiments were carried out, but the results were not entirely satisfactory, as it was found impossible to complete the experiments quickly enough to avoid partial decomposition and corresponding loss in activity. The final solutions, therefore, always contained more or less inactive material formed during the progress of the experiments themselves. Several series of solubility measurements were carried out, nevertheless, as rapidly as possible and at 6° C. They were disappointing in that they indicated clearly that the preparation was a mixture. To confirm this result a study was made of the changes in activity when the protein is denatured, as was done with pepsin, except that in this case denaturation was carried out by heating in dilute acid. The trypsin protein when treated in this way becomes denatured and insoluble. This experiment showed clearly that the preparation, although crystalline, was undoubtedly still a mix-

ture, since a considerable amount of the protein could be coagulated and removed from solution without decreasing the activity of the solution. As the heating was continued, however, and more and more insoluble protein was formed, it was found that the activity began to decrease about in proportion to the formation of insoluble protein. It appeared, therefore, that the original preparation contained two proteins, one of which was easily coagulated by dilute acid and carried no activity with it, while the other one was much more resistant to acid and was associated, at least, with the activity. These results furnished also a further method of purification since, by heating the crystalline material in dilute acid, about one third of the protein could be removed without loss in activity. Considerable amounts of the preparation were treated in dilute acid in this way and a second preparation obtained which was about twice as active as the first one. It crystallizes more readily than the first preparation and the crystals are similar. The purity of this material was again tested by solubility measurements and the results were more satisfactory than with the first preparation but still not really convincing, owing again to the very unstable nature of the substance. The loss in activity when a solution of this substance was heated in acid was just proportional to the amount of native protein changed to denatured.

The protein is rapidly digested by pepsin and several careful experiments were done in which the amount of trypsin protein digested by pepsin was compared with the loss in activity. They showed very clearly that digestion of the protein with pepsin resulted in the loss of a corresponding percentage of the activity, so that whenever a molecule of the protein is digested by pepsin it loses its tryptic power. There is, then, no evidence that the products resulting from the action of pepsin on trypsin have any tryptic power. The experiments were varied by allowing the preparation to digest itself in dilute alkaline solution. Under these conditions also the decrease in the protein concentration is exactly parallel to the decrease in the activity of the solution.

It was found by Mellanby and Wolley that trypsin solutions possessed the remarkable property of retaining their activity after being heated nearly to boiling for a short time in dilute acid. The solutions of crystalline trypsin may also be heated for a short time nearly to boiling without any loss in activity and, what is still more remarkable, without the formation of any denatured protein. This result is obtained only if a solution is allowed to cool before being tested for either denatured protein or activity. If the solution is tested while still hot, it is found that the protein is all denatured and, in addition, that the solution is inactive. It is possible to show, therefore, that the formation of denatured protein is accompanied by a loss in activity and, what is more significant, that the reformation of soluble, native protein from the denatured protein is accompanied by recovery of the corresponding activity. As in the case of pepsin, therefore, it is found that whenever anything is done to the protein molecule the activity is lost and that, on the other hand, when the denatured, inactive protein is changed back into soluble, native protein the activity is regained. If it be assumed that the activity is due to some special active molecule, then it must be assumed in addition that the conditions for inactivating these hypothetical molecules must be the same for denaturing the protein molecule and also that the conditions for rendering the hypothetical molecule active again are precisely the same as those for forming native protein from the denatured protein. The behavior of proteins in general is so peculiar and characteristic that it is extremely unlikely that any other type of molecule would be affected in the same way and to the same extent so that the possibility that the activity is due to a non-protein molecular species present appears very remote. It is possible, on the other hand, that the preparation is a mixture or solid solution of several closely related proteins and that only one of these is active.

The general properties of pepsin and trypsin which have been determined by these experiments show that they are similar in many respects to hemoglobin. Their peculiar ability to digest proteins is lost as soon as any change, such as denaturation, is made in the molecule. The denaturation of hemoglobin likewise results in complete loss of its characteristic property of combining reversibly with oxygen. On the other hand, some of the properties of hemoglobin, such as its combination with carbon monoxide and its characteristic absorption spectrum, are retained by the denatured form and even to some extent by pieces of the molecule when it is hydrolyzed. In the case of pepsin and trypsin there is, at present, no indication that any of the pieces of the molecule retain their digestive power; but it is quite possible that more careful search would show more or less activity associated with one of the decomposition products. The peculiar properties of hemoglobin are known to be due to the presence in the molecule of a characteristic group which differentiates it from other proteins. It is quite possible that the enzyme proteins likewise contain a characteristic group, but so far no evidence has been found for its existence. They are, however, quite different from other known proteins in many respects and this difference must be due to some characteristic difference in chemical structure.

(This article is based upon a lecture presented at the Marine Biological Laboratory on August 18).

THE BIOLOGICAL LABORATORY

COLD SPRING HARBOR

ELECTRIC EXCITATION IN NERVE

Kenneth S. Cole

When the external potential difference between two points of a nerve is changed, the nerve may be stimulated and propagate an impulse. The magnitude of the electric field which is just sufficient to stimulate depends upon:

(1) the angle between the axis of the nerve and the direction of the field,

(2) the length of nerve which lies in the field,

(3a) the length of time for which the field is applied, or in general,

(3b) the manner in which the field varies as a function of time.

Effect of Direction and Extent of Electric Field

It has long been known that an electric field perpendicular to the nerve axis is very inefficient as a stimulus but the early quantitative measurements of the dependence on the angle were not entirely satisfactory. The work has been repeated by Rushton, 1927, under carefully controlled conditions. The uniform field strength E (of a constant duration) which was required to excite a constant length of nerve in a large body of saline was measured as the angle θ between the direction of the field and the axis of the nerve was varied. Within experimental limits it was found that $E = E_o/\cos\theta$ where $E = E_o$ when $\theta = O$. This means that only the component of the field in the direction of the nerve is effective for excitation.

In the same paper, Rushton, 1927, a relation is derived between the exciting field strength E parallel to the nerve and the length of nerve x exposed to the field. For this purpose the nerve is considered as an insulated cable having a relatively well-conducting core in which the current is longitudinal and a relatively non-conducting sheath where the current is radial. The maximum current density is found under the electrodes and it is assumed that excitation occurs when this reaches a definite fixed value at the cathode. This is equivalent to a liminal potential difference across the sheath since the resistances are assumed constant. It is then found that

$$E = E_o/(1 - e^{-x/\lambda})$$

where $E = E_o$ when x is large and λ is the length of nerve for which the radial sheath resistance is equal to the longitudinal core resistance. Although the older results are open to question they follow the general form of this equation, and Rushton's improved technique gives data which agree very well with the theory. As far as electric fields of constant duration are concerned, we may feel justified in accepting the hypothesis that excitation occurs when a liminal potential difference is established across the sheath, or membrane, of the nerve.

Effect of Duration of Electric Field

When the duration of the electric field is varied, the problem becomes considerably more complicated. DuBois Reymond, 1848, stated that the excitation was a function of the time rate of change of the current density. Fick, 1864, showed that this was only partially true and that for rectangular pulses, in which the current rose and fell practically instantaneously, the duration was a factor. Since that time a relation between intensity and duration of stimulation of the type shown in Fig. 1 has been found for almost every known irritable tissue. Even for a very long duration, excitation will never occur when the intensity of stimulus is less than a certain value which Lapicque, 1926, has named the *rheobase R*. The duration of a just effective stimulus having twice the intensity of the rheobase has been called the *chronaxie* γ, also by Lapicque.

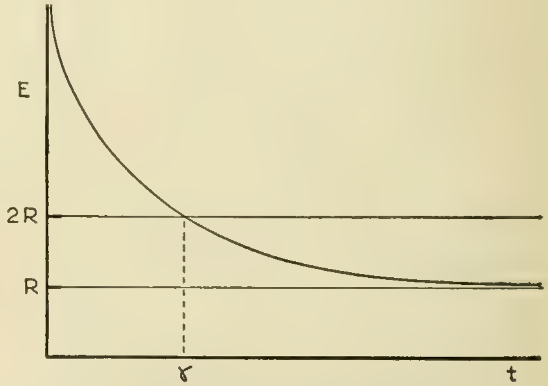

Fig. 1. Diagramatic relation between the duration t of an effective stimulus and its intensity E. R is the rheobase and γ is the chronaxie.

The first analytical expression for this curve given by Hoorweg, 1892, for a condenser discharge stimulus, and by Weiss, 1901, for a rectangular pulse, is

$$E = R\ (1 + \gamma/t) \qquad (1)$$

When the effective intensity is expressed as a current, the quantity of electricity delivered by an electrode is a linear function of the time. Experiments over an extended range of durations show systematic deviations from this simple law.

Condenser Models, Rectangular Pulse

In 1907, Lapicque considered the nerve sheath as a condenser *C* with a leak of resistance r_1 and represented the external and internal resistances by r_0.

If excitation occurs when the potential difference across the membrane reaches a definite value, the relation between the intensity *E* of the applied rectangular pulse, and the duration *t* for excitation is given by

$$E = R/(1 - e^{-t/\tau}) \qquad (2)$$

where the rheobase is *R* and the chronaxie $\gamma = .69\,\tau$. When t/τ is not too large, this has the form of Weiss's law, equation (1). Hoorweg's postulate that the rate of increase of excitability

$$\frac{dp}{dt} = \propto E\ e^{-t/\tau}$$

gives the above result with a rectangular pulse if $p = \propto \tau R$ for excitation.

Recently Ebbecke, 1927, and Hill, 1932, have extended Lapicque's concept by considering specifically the inside and outside resistances and the sheath condensers under each electrode with a "resting" potential in series with the leakage resistance. This circuit can be reduced, Cole, 1928,

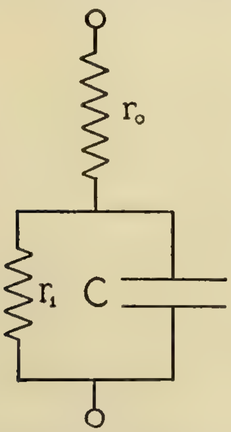

Fig. 2. Equivalent circuit of condenser nerve model. r_0 r_1 are resistances and *C* is a condenser.

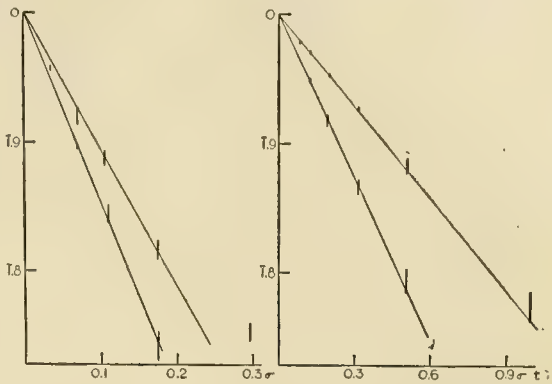

Fig. 3. Plot of Rushton's, 1932, data after Hill, 1932, on basis of condenser nerve model. Ordinates are log $_{10}$ (1—R/E) and abscissae are durations in σ. Left is for warm nerve and right for cold.

to that of Fig. 2 and gives equation (2) for excitation by a rectangular pulse.

On the other hand, Blair, 1932, has disregarded structure and postulated the excitability *p* to be increased at a rate proportional to the stimulus and to be decreased at a rate proportional to the change in excitability or

$$\frac{dp}{dt} = KE - k\,p$$

Excitation occurs when *p* reaches a certain value and for a rectangular pulse equation (2) is arrived at.

Since these theories give the same equation, the data can at most only decide whether a condenser nerve model is satisfactory or not. Blair, 1932, has analyzed a considerable amount of Lapicque's, 1931, a, b, recent data from this point of view and finds that it fits rather well except for a constant which seems to depend upon the electrodes. Hill, 1932, has plotted log (1 — R/E) vs. t from Rushton's, 1932, data, as shown in Fig. 3, to support the theory. Here Blair's constant term is assumed to be zero. Hill accounts qualitatively for the effect of electrode size, electrode separation, and fiber diameter, and calculates a membrane 2.2 μ thick having a capacity of $1.6 \cdot 10^{-3}$ μ f. per sq. cm. and a specific resistance of 10^9 ohm cm.

Diffusion Polarization Models. Rectangular Pulse

In 1908, Nernst published a theory of nerve excitation which has had a profound influence on the field. He assumed that the nerve sheath was a semi-permeable membrane which allowed only ions of one sign of charge to pass through. When

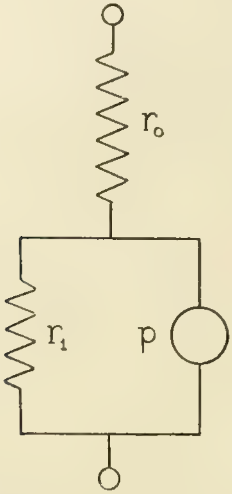

Fig. 4. Equivalent circuit of general polarization nerve model. r_0 r_1 are resistances and p the polarization element.

an electric current flows, the permeating ions carry only their normal share of the current in the electrolyte on each side but must take the whole burden inside the membrane. This means that they must move into one side of the membrane faster than they are normally delivered up by the solution, while an excess is created in a similar manner on the exit side. Ions of both signs sent to or from the membrane surfaces by diffusion tend to keep the changes from proceeding too rapidly with the result that the concentration change at the surface $\Delta c = Ki\sqrt{t}$ where i is the current density and t the time it has been flowing. For small concentration changes, the counter electromotive force produced is also proportional to $i\sqrt{t}$. Nernst then assumed that when the concentration had changed by a certain amount (or the polarization had reached a certain value) excitation took place and

$$E = K'/\sqrt{t}. \qquad (3)$$

For cells of small dimensions, this relation can only be expected to hold for short times, but Hill, 1910, extended the theory to include the interaction between two membranes so that it might be expected to hold for longer durations. The simplified expression for a rectangular pulse is

$$E = R/(1 - \mu\,\theta^{t}) \qquad (4)$$

Strange as it may seem, μ is nearly unity for many cases and then equation (4) the same as equation (2).

Lapicque has met this failure of the Nernst theory at long durations and provided a rheobase by an empirically determined "canonical curve" which expresses a large amount of his data,

$$E = R\sqrt{\frac{t + \theta + \sqrt{(t - \theta)^2 + 0.16\,\theta^2}}{2t}}$$

where θ = 3.8 times the chronaxie.

It should be pointed out that in order to obtain a rheobase it is only necessary to postulate that the diffusion polarization p is but one element of a circuit which may be reduced to that of Fig. 4, that is, it replaces the condenser of Fig. 2. The potential difference across p can never exceed $r_1 E/(r_1 + r_o)$ and it is to be assumed that this must reach a liminal value for excitation to occur.

Cremer has recalculated the data of Weiss, 1901, and finds that it agrees well with the Nernst theory when the duration is not too long. Lapicque, 1926, has proposed the canonical curve as the best generalized expression of his data and it reduces to the Nernst equation for durations of a chronaxie or less. Rushton, 1932, on the other hand, finds that the canonical curve does not fit his data on the frog sciatic. In order to visualize this, Rushton's data have been plotted in Fig. 5 in the form log E vs. log t. It is seen that for times less than a chronaxie the data are expressed by

$$E = Kt^{-\alpha}$$

(except for the same point which Hill believes to be "obviously in error" in Fig. 2 above). α has the value 0.76 for warm nerve and 0.86 for cold, instead of 0.5 as required by Nernst and Lapicque. Ninety-nine of Lapicque's experiments show an average value of α = .656 with a "standard deviation" of .22, Wegel, 1932.

It is thus impossible to make a catagorical decision between the condenser and the diffusion

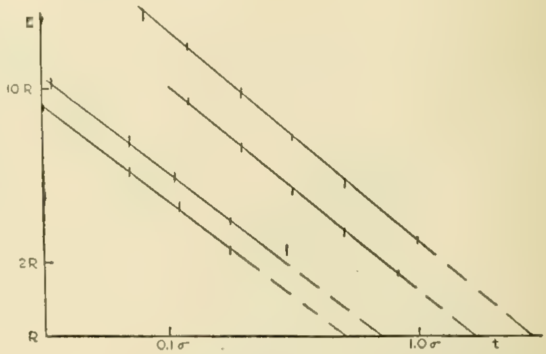

Fig. 5. Plot of Rushton's, 1932, data on logarithmic coordinate scales. Ordinates rheobase units and abscissae are σ.

polarization hypotheses on the basis of rectangular pulse excitation. Some data fit one and some fit the other, and it is wise to look at other phenomena.

Condenser Discharge Pulse

Hoorweg, 1892, made extensive use of condenser discharge stimuli and the method is so simple that it has had wide application. Since the form of the condenser pulse is so different from the rectangular pulse it might be thought that the predicted intensity-duration curves might be quite different for the condenser and diffusion polarization nerve models. When Δ is the "time constant" of the condenser stimulus which corresponds to the duration of a rectangular stimulus t of the same maximum intensity, it has been shown that for the condenser model

$$t = 0.3466\ \Delta \qquad \text{Blair, 1932,}$$

while for the Nernst model

$$t = 0.344\ \Delta \qquad \text{Eucken and Miura, 1911.}$$

It seems quite impossible to determine this factor accurately by experiment—as is also shown by both hypotheses—and Lapicque's value 0.37 certainly has no decisive value.

Sub-threshold Excitability

Before consideration of the other excitation phenomena which involve, in general, multiple stimulation it is well to discuss a very important result of Bishop, 1928, and the extended investigations of Erlanger and Blair, 1931, a, b. Bishop measured the excitability p of a nerve at different intervals t after the application of a sub-threshold constant potential by means of a very short induction test shocks, with the result shown diagrammatically in Fig. 6. If it be assumed that the effect is linear and reversible, we can immediately explain most of the electrotonic effects by Fig. 7,

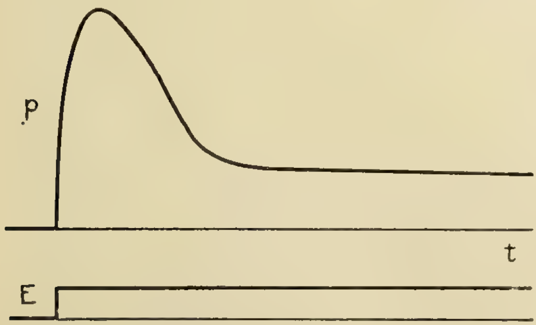

Fig. 6. Excitability p at cathode as a function of time t during an inadequate direct current stimulus E (Bishop, 1928).

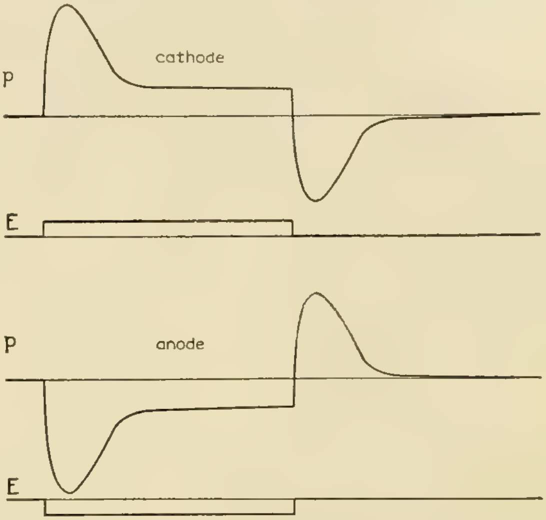

Fig. 7. Idealized excitabilities p at cathode and anode for make and break of inadequate stimulus E to be predicted from Fig. 6. See Erlanger and Blair, 1932.

which are indeed the idealized results of Erlanger and Blair. As the duration of the stimulus is made very short we should find that the excitability

$$p_i = E \cdot \frac{d\, p_o\,(t)}{d\, t}$$

which is again found by Erlanger and Blair. For linearly increasing strength of stimulus

$$p_i = E_o \int_0^t p_o\ (t)\ d\ t$$

which does not agree as well as might be hoped for with the results of Lucas, 1907, and Erlanger and Blair, 1931. Such treatment seems to be rather hard on the nerve and may involve still more complicating factors. It seems evident, however, that the explanation of Bishop's curve will automatically solve many of the questions raised by the use of more than a single pulse for a stimulus, and furthermore that the initial rising portion of this curve should explain the single pulse excitation. Bishop has proposed a complex resistance-capacity model, but Erlanger and Blair find that at low temperatures the maximum of the excitability curve is quite flat and starts downward at a finite rate instead of gradually. This suggests that an adaptation mechanism starts into action at what would be approximately the end of the absolute refractory phase for a stimulated

nerve,—when the propagated impulse is well under way.

Since none of the excitation theories (with the possible exception of Hoorweg's) allow of this decrease from the maximum of excitability for an inadequate constant potential stimulus, and since there are experimental indications that this decrease may be due to another mechanism, it seems unwise to attempt to apply either the condenser or the Nernst model to other than single pulse excitation.

Alternating Current Excitation

Blair's, 1932, hypothesis and the condenser model both show that for alternating current (repetitive) stimulation

$$E^2 = R^2 + K^2 \omega^2$$

where $\omega = 2\pi n$ and n is the frequency, while the simple Nernst model gives

$$E = R\sqrt{\omega}$$

There are no experiments which completely substantiate either equation (Asher, 1923, Krüger, 1928, Renquist and Koch, 1930, Blair, 1932) over a wide frequency range. This is not surprising in view of encroachment into the relative and absolute refractory phases to be expected at high frequencies and the effect on the relative refractory phase of a stimulus placed in the absolute phase as found by Erlanger and Blair. It should be suggested, however, that the rather abrupt changes in K and R found at different frequencies by Blair may well be due to the combined effects of the refractory periods resulting in both a change of apparent threshold, and excitation at less than the stimulating frequency.

It now becomes reasonable to assume that only the excitation data for single pulses come within the scope of the two types of theory, and even then it seems impossible to generalize without independent supporting evidence.

A Generalized Hypothesis

If it is allowable to assume that excitation occurs when a counter-electromotive force reaches a liminal value and that, without excitation, the magnitude of this counter e. m. f. p at a specified time, t, after the constant current, i, has started to flow, is proportional to the current—then we may consider it in terms of a resistance $r(t)$ which varies as a function of the time. That is, $p = i \cdot r(t)$. It is then only necessary to put p in a network of the type of Fig. 4, or its equivalent, and compute its over-all resistance as a function of time. By comparison with experimental data it should be possible to determine $r(t)$. Unfortunately the mathematical problem in this form is not simple and it is only recently that measurements of this type have been made. There is, however, another possibility since a variable resistance of this type on direct current will be equivalent to a resistance and a reactance (capacity, or inductance) when measured with alternating current of a certain frequency. In general, both the resistance and reactance will change with frequency, for this is merely on alternating current application of the Fourier integral equation. When live tissues are measured with alternating current at various frequencies, it is found that they give the same results as a single element p having in series a resistance r_p and a capacity c_p, both of which vary with the frequency n. It is furthermore found, Cole, 1932, that for many tissues this element gives $r_p c_p \omega = m$ where $\omega = 2\pi n$ and m is a constant, independent of frequency. It is then said that the variable element p has a constant "phase angle". By a Fourier integral analysis which is the direct inverse of that employed by Fricke, 1932,—since we wish to calculate his assumption from his answer—it is found that

$$r(t) = K' t^{\propto} \qquad (5)$$

where $m = \cot(\propto\pi/2)$ and K' is a constant. Thus if m is a constant and known we can immediately calculate $\propto$, which will lie between zero and one.

From the data of Lullies, 1928, on frog sciatic nerve, it can be shown that m is constant over a considerable frequency range and has the value 0.49, Cole, 1932. Then $\propto = 0.71$ and we would suspect that if Lullies had determined the excitation curve he would have found $E = Kt^{-0.71}$ when t was less than a chronaxie. Since these data are not available, we note that Rushton's exponents were 0.76 and 0.86 for warm and cold nerve respectively, while over 60% of the exponents quoted by Wegel, 1932, lie between 0.6 and 0.8.

The few biological materials for which the alternating current data have been computed give values of $\propto$ ranging from 0.6 to 0.8, with the exception of red blood cells where $\propto = 0.9$. The condenser model requires that $\propto = 1.0$ and the Nernst model that $\propto = 0.5$. Although the alternating current data are far from conclusive, the indications are that nerve lies in the region included by the tissues examined up to the present, and that this region is intermediate between the condenser and polarization membrane models.

It is, therefore, suggested that many of the active and passive phenomena of living tissues may depend upon a membrane polarization having a

variable resistance to direct current of the form of equation (5).

Summary.

It is commonly assumed that a nerve fiber has a core of comparatively good electrical conductivity with a sheath of much lower conductivity and that if electric excitation is to occur, a liminal potential difference across this sheath must be created. The relation of the threshold stimulus to (1), the angle between the nerve and the stimulating field, and (2), the length of nerve exposed to the field, may be explained on this basis.

It has further been commonly assumed that the sheath undergoes a change in polarization as the result of current flow and this has been postulated as due to either (1), a pure static capacity, or (2), a diffusion polarization at a semi-permeable membrane. Neither of the theories developed on these two hypotheses has exclusively explained single pulse excitation data satisfactorily. While they both fail to explain the decrease of excitability in sub-threshold direct current stimulation, there is a possibility that this effect may result from a distinct recovery mechanism. The theories should not then apply to more than the initial rise of excitability and it is not to be expected that multiple stimulation phenomena can lend support to either.

Alternating current measurements of the resistance and reactance of nerve and other tissues suggest that the polarization of the sheath is neither that of a static capacity nor a simple diffusion, but intermediate between the two. Applied to the excitation of nerve, this leads to a strength-duration relation of the form

$$E = K t^{-\alpha}$$

when the time is not too large. From alternating current data on one nerve $\alpha = 0.71$ while from a recent set of excitation data $\alpha = 0.76$.

Thus alternating current measurements do not support either the condenser or the diffusion polarization models but predict a type of polarization which has some support from excitation data.

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Discussion

Dr. Blinks: Would you introduce a chemical reaction or metabolic element—in addition to the purely physical effects such as polarization and diffusion—to account for the deviations of the excitability curve from the theoretical forms? There is apparently such a metabolic response in the plant cell which follows the production of alkalinity by an external medium. When ammonium salts are applied to *Halicystis* there is a tendency for the potential to give a cusp and then recover as if the cell responded by increased acidity. The same can be observed with current flow. Of course, we can say nothing about the relations in nerve from the case of plant cells where it is a matter of seconds or even minutes in the case of ammonium application.

Dr. Cole: I have no concept of the adaptation or recovery portion of the curve. The results of Erlanger and Blair at low temperature suggest that this phase may start in very abruptly for inadequate stimuli and be linked with the relative refractory phase of an adequate stimulus. It would then seem reasonable—and considerably simpler—to ascribe it to a delayed process not directly connected with the rise of excitability which is under theoretical consideration.

Dr. Gasser: There is one theoretical difference between the Nernst and condenser hypotheses which may be put to experimental test. According to the Nernst formula the energy of the current necessary for excitation is constant, whereas it follows from the exponential formula that at some value of the time the energy runs

through a minimum. In practice there is a current of minimum energy.

Dr. Cole: I have not worked out the energy matter and the discussions which I have seen are not entirely convincing. For example, the energy has sometimes been computed for a constant current rectangular stimulus as proportional to i^2t, which leads to the constant energy for the Nernst model and the minimum for the condenser model. We are concerned with the energy delivered to the nerve

$$W = \int_0^t e\,i\,dt$$

which reduces to the above simple form when the resistance of the nerve is constant but may not do so otherwise. It is also possible that there is a difference between constant current and constant voltage stimuli, so one must also be sure that the assumptions of the theories are satisfied by the experiments.

Dr. Müller: How large is the threshold value of an electric field of long duration?

Dr. Cole: The rheobase for frog sciatic as usually set up is less than 100 mv.

Dr. Müller: Is it not possible that the current and not the voltage determines the excitation? The fact that a very slowly increasing voltage does not produce any effect might be due to the production of counter e m f s which can develop during a slow increase of current, but which have no time to establish themselves if the voltage increase is fast.

Dr. Cole: It may be useful to take such a viewpoint—particularly for the falling portion of the excitability curve. The phenomena of linearly increasing and multiple stimuli are not handled by the theories in which it is assumed that the excitation is caused rather than hindered by the counter e m f.

Dr. Gasser: Is there only one curve of excitation? The data which fit the canonical curve do not fit at all well to the exponential formula.

Dr. Cole: It is probably too much to hope that all irritable tissues will fit the same formula but there seems to me to be a decided possibility that many tissues will polarize according to a law t^{δ} and have values of δ which lie in a limited range—say from 0.6 to 0.8. It may then be difficult to distinguish the lower and upper ends of the range from the Nernst and the condenser formulae respectively.

Dr. Blinks: How do you consider the evidence connecting chronaxie with the speed of propagation?

Dr. Cole: I know very little of the evidence, but I understand it has been found recently that the relationship is not as simple as had been previously supposed.

Dr. Shedlovsky: Over what frequency range has the phase angle been found constant?

Dr. Cole: Lullies measured the resistance and reactance of nerve from 28.8 cycles per second to 332,000 cycles per second. His data indicate a constant phase angle element which predominates except at the two highest frequencies, 48,500 cycles per second and 332,000 cycles per second.

Dr. Fricke: In only very few cases do we find the polarization capacity of cells to vary as the inverse square root of the frequency. Usually the variation is less fast but increasing as the frequency is increased.

AXON ACTION POTENTIALS IN NERVE

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Physiologists record the potentials generated in nerve during activity for a number of reasons. The primary one is to obtain the form of the potentials themselves, as the electrical sign of activity is the only one which can be located with any precision. Heat measurements, ingenious as they are, are still under distinct limitations in their ability to reveal the times at which the heat is evolved; for instance, they show very little of the cycle which must obtain in the course of a single impulse; and chemical studies only bring to light the stable end products of metabolism and tell nothing whatever about when the catabolites are formed. The potential curve being thus the one record of action which gives definite information as to *when* events occur, it becomes a frame of reference for possible correlations with other signs of activity and for comparison with irritability changes (the time course of the latter may be determined with the precision necessary to make such a comparison possible). Furthermore, action-potentials may be used for the tracing of impulses through the nervous system and for analysis of nerve fibers into their components with the idea of associating the parts with the functions they subserve.

In all the foregoing enterprises the electrophysiologist is on solid ground, but he is not satisfied to confine himself within these limits of safety; we often find him in the hazardous occupation of inferring from the potentials, themselves, what the processes behind them may be. Just how far this is from sound practice follows from but a cursory consideration of the precautions taken by a physical chemist in the measurement of a potential. In the first place the physical chemist starts out with pure chemical substances of known concentration. If a chemical reaction be involved, its nature is known and the reaction itself is chosen because it meets the necessary exactions imposed with respect to reversibility. The cells are set up in such a way as to avoid unnecessary diffusion potentials and the readings are made with the cell so balanced that it is supplying no appreciable current. None of these conditions is fulfilled when a lead is made from the surface of a nerve; the source of potential is unknown and no matter what precautions may be taken against the drawing of current into the circuit in which the nerve is placed, the damage is already done. The seat of production of the potential is imbedded somewhere within the nerve and must set up currents of an undetermined nature in the inactive portions of the tissue. These can hardly fail to be without repercussion on the source; in fact, Lillie, in his theory, makes very definite use of the current in the local bio-electric circuit as an aid in restoring an active portion of a nerve to its normal resting state. Furthermore, in the absence of a knowledge of the resistance of either the source or the shunting resistance, it is quite impossible to know what relation the potential recorded has to the actual working potential. On account of this unsatisfactory situation but little attention has been given to the absolute value of recorded potentials and all the interest has centered around the time at which they occur. The magnitudes can be of use only in comparison of the same fibers in a given nerve under two conditions of the environment in which the unknown and uncontrolled variables may be considered to be sufficiently constant.

From the foregoing remarks it can clearly be seen that it would be to the advantage of every electrophysiologist to nail this notice on an imaginary laboratory door—*you cannot determine a process from a potential.* It is not to be expected that attempts to do so would be thereby prevented, but it would be done in the hope that the products of such attempts would at all times be recognized for what they are: a form of inspired guesswork. Now the general subject matter of this symposium indicates that the interest of the participants is focused primarily on the nature of the potentials rather than on their workaday applications; that is, on just that aspect of the subject on which it is most difficult to supply information. When confronted with a problem which has no definite answer, the only course open is to state the problem definitely so as to have clearly before one the phenomena which demand explanation; and this I shall proceed to do. Such theoretical considerations as suggest themselves will be left to the end.

For the understanding of what is to follow it is necessary to mention two points in connection with the technique of making a lead. What is desired is the change of potential with respect to time at some point, as A (fig. 1), on the surface of the nerve and an appropriate electrode is located at that place. The difficulty comes in connection with the second lead which is necessary for the completing of the circuit. If the potential change is to be recorded without distortion, the potential at the second lead must not change. In practice it is of no avail to place the second lead off at a distance from the nerve, because that in effect only provides a diffuse connection with

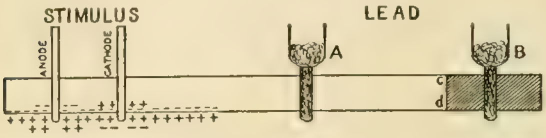

Figure 1. Diagram of the position of the electrodes on a nerve when a "monophasic" lead is recorded. The theoretical movement of ions is indicated in the region polarized by the stimulating current.

the nerve's surface and the sum of all the potential-changes led off from the surface does not add up to zero. If the second lead be placed on the surface of the nerve the two leads are alike and one obtains only the momentary difference between them. So the routine procedure is to destroy the nerve under one electrode (B) by heating or crushing and thus prevent the impulse from penetrating to this region. In theory the plasma membrane is now destroyed and the lead from electrode B is continued by way of the cytoplasm in the interior of the intact portion of the nerve to the inside of the plasma membrane in the region of A. In actual practice the procedure nearly always fails to achieve the desired result. Even after a fresh crush or heat coagulation some of the nerve's activity is led off by way of electrode B, producing what is known as the diphasic artifact, and, as the preparation stands, the lead becomes progressively greater. This is usually explained as due to the re-establishment of some degree of polarization at the dead-live junction, so that the end of the nerve can undergo a potential change (at the line c-d) which can be effectively led off by the dead portion of the nerve which is now applied in the direction normal to the surface. The most effective methods of obtaining monophasic leads employ potassium salts or cocaine, the one method permitting a maximum demarcation potential, the other none at all or even a positive one[4]. It is difficult to see what these differently acting substances have in common unless it is the prevention of formation of a surface film at the end of the nerve. Potassium salts destroy the surface film and by their continued presence must prevent its reformation. Cocaine probably produces a block by an action at the surface of the fibers, and it is quite possible that the interior is so little affected that there is no tendency of the protoplasm of the unnarcotized portion to wall itself off from the narcotized part. Even the last two methods often fail to free the nerve entirely from signs of diphasicity. This may be due to the fact that a dead continuation of the nerve is not a perfect end-on lead to the intact portion but that some twigs of current can run from the surface next to the ends by way of salt solution in the spaces between the fibers, in the sheath, and on the surface. Another method of obtaining a monophasic lead is to place the electrode A close to c-d so that the diphasic artifact is almost directly under the first phase. The result is then to depress the height of the latter rather than to distort its form. Only limited application of this method is possible, however, because the spike cannot be recorded beyond the point at which it still reaches full magnitude; and the method is useless for after-potentials.

The second point in connection with the technique of leading arises from the fact that nerves are not homogeneous in their composition. They are made up of fibers with and without myelin sheaths and having diameters ranging from 20μ downward. If a nerve be stimulated with a strong very fast induction shock so as to cut the utilization and latent periods to a minimum, all the fibers will become active under the stimulation cathode at the same time, but they will be conducted along the nerve at very different rates, there being in the neighborhood of 100-fold variation between the fastest fibers and the slowest. The result is that, if a lead be made at a distance from the stimulus, the impulses undergo considerable temporal dispersion and the potential picture, which is the sum of the potentials in the individual fibers, fails to show the form of the potential as it exists in any one of them (fig. 2). To obviate this difficulty several procedures are open. One of the best is to lead directly from the stimulating cathode with the employment of very fast induction shocks weak enough to stimulate only a portion of the more irritable alpha fibers. There is then no temporal dispersion and the conditions as to homogeneity of material, utilization period of the shock, and latency, introduce no serious difficulty. Another method is to use very weak shocks and considerable amplification. The fibers entering into the formation of the potential are then so homogeneous in their qualities, and their velocities of conduction so much alike, that conduction may be permitted with the occurrence of only negligible temporal dispersion. This method frees the front of the wave from the shock artifact but is not so useful for the low potentials which come at the end of the response. Carried to the limit the method can be made to reveal activity in single fibers. This is not necessary for many problems and when it is tried, new forms of distortion are encountered which are not inherent in the high amplification involved. In order to differentiate the action potential as it exists in any one fiber from the composite picture obtained from a mixed nerve it is designated as the axon action potential.

Subthreshold phenomena. In the short interval between the start of a potential applied to a nerve for the purpose of stimulating it and the beginning of the actual disturbance which will be propagated, there is ample evidence that much is going on. The subject has been studied most recently and in greatest detail by Erlanger and Blair, the method being to apply a conditioning potential at an intensity below threshold and at various intervals thereafter to test the irritability with a fast induction shock. The value sought is that of the smallest shock which will bring the excitation process to threshold. When the conditioning shock is also an induction shock, the irritability immediately rises and for a period of about 0.5σ (frog nerve at room temperature) excitation may be brought to threshold with a weaker shock than is necessary for resting nerve (summation interval). Then the irritability falls below normal (depression phase). It reaches a minimum at about 1σ, after which it returns toward normal over a period of 4σ or longer—a duration very similar to that of the refractory period which would have supervened had the excitation gone above threshold.

The interesting feature of this cycle of irritabilities which is set up by a subthreshold shock is that there is nothing to parallel it in the electrical potential picture. If a lead be made from the stimulating cathode, all that is obtained is the well-known disturbance which is called the shock artifact. It is occasioned, among other things, by the polarization which is produced in the nerve structures and shows as a decremental curve which usually falls to half-value in well under 0.5σ. Neither in form nor duration does it have any relation to the irritability curve. If the temperature of the nerve be lowered the cycle of irritability changes is considerably prolonged, while the temperature coefficient of the artifact is so nearly unity that it seems to be so in actual measurements. At the anode the irritability curve is the mirror image of that at the cathode[7].

Figure 2. Form of the action potential when conducted impulses are led from the sciatic nerve of the green frog. Single sweep recorded on a cathode ray oscillograph. Temperature 27° C. Conduction distance 48 mm. The time is marked in σ. The first deflection is the shock artifact; the time between it and the spike is the conduction time. The spike shows α, β, and a small γ wave.

If the conditioning shock be a rectangular current applied at a subrheobasic level, the irritability rises for a time, reaches a maximum which may be a plateau, then falls off again. The curve gives evidence that two processes are in operation, one acting to increase irritability, the other to decrease it. The latter is brought out distinctly when the current is broken for the level of irritability then passes quickly into a depression phase[7]. As with the induction shock there is no potential sign of this irritability cycle. Even when the conditioning current is 95 per cent. of rheobasic strength, the electrical picture gives no hint of the potential change which would occur if only the remaining 5 per cent. of the threshold strength were added to the current.

The spike. When the rectangular current is strong enough there appears quite abruptly, after a period of utilization which depends upon the strength of the current, a typical self-limited transient disturbance. This is the "action current" of the older literature, but it will be referred to as the "spike potential" to differentiate it from other potentials which also occur. The periods of utilization plotted against the strength of the current give the characteristic time-strength curve of excitation. Bishop[3] has shown that the curve may be predicted, at least as a first approximation, on a polarization basis, the times being determined as those at which a constant amount of polarization would occur at the several strengths of current. It is thus made apparent that a definite amount of work must be performed upon the nerve before the spike is, in effect, released—we are dealing with a process which has the appearance of trigger action. Once started the spike develops on an all-or-nothing basis; both its magnitude and time course are determined by the nerve itself and not by the manner in which the impulse is set up.

The front of the spike is an S-shaped curve in which the maximum slope is attained very early. Following the spike crest (0.3σ is a value for the crest time often seen in frog nerve), the decline of potential is slower than its rise. Restoration to within 5 per cent. of normal occurs in 3 crest times; then the decline becomes more gradual, and what appears to be spike may be traced as long as 20-25 crest times[8]. It is at the bend in the curve at about 3 crest times that the nerve is first able to give a second response. The relative-

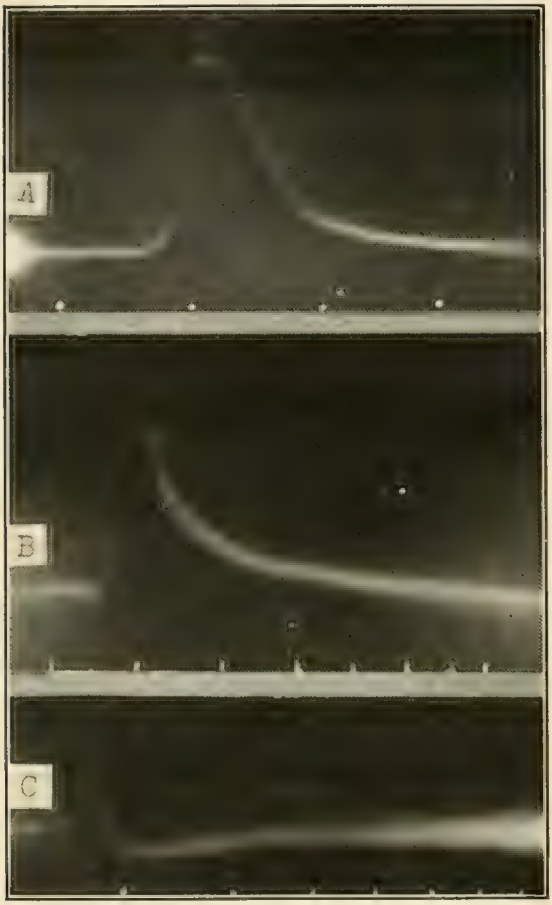

Figure 3. The parts of the axon action potential of the green frog sciatic nerve. The deflection is upward when the active electrode (*A* fig. 1) is negative.

A. Spike. Record of the most irritable fibers only (a fibers) made after 4 mm. conduction; it gives approximately the form of the axon spike. Temperature 20°C.; time marks, 1σ.

B. Negative after-potential. Fresh nerve at 23°C.; time marks, 10σ. Note that the potential ends by crossing the zero line.

C. Positive after-potential in a fresh nerve with a very short negative after-potential (30σ). The positive after-potential has a maximum value of 12 microvolts and amounts to 0.08 per cent. of the crest height. Time marks, 100σ.

ly refractory period begins at that point and is usually considered as having no potential sign, but it is possible that a correlation may be found with the tail of the spike.

Throughout the general run of laboratory procedure the spike maintains its form with great fidelity. Unbalancing the ionic environment of a nerve by the addition (or subtraction) of ions of the alkali metals or the alkaline earths (Graham) or of hydrogen ions only produces a change in height, usually a decrease. The same is true of asphyxia and drugs of the veratrine group. The one variable to which the spike is distinctly susceptible is temperature; cooling causes a prolongation accompanied by a considerable falling off in height. The Q_{10} of the duration is about 2 in the region of 20° C., and it increases progressively as the temperature is lowered.

All the spikes in a mixed nerve are not alike. Three types can be readily identified: the A type which has already been described, the B type lasting ten times as long, and the C type with a duration more than seventeen times as great; but, in the light of the experiments recently reported by Blair and Erlanger, one can no longer consider the spike durations as completely described in three groups. These authors have examined the potentials in single fibers and found a continuous range of durations. The duration is nearly constant in most of the A range, as previously held; but it begins to increase in the slower A fibers and increases more rapidly below this point. From the new findings it follows that the existence of the major elevations in the action potential from mixed nerve are due to the predominance of fibers of certain types rather than to the existence of sharply differentiated kinds of fibers.

After-potential. The disturbance which makes up the total of a single nerve response ends with a long negative potential of low magnitude, followed by another longer and lower potential with a positive sign (fig. 3, *B* and *C*). The negative potential, which is called the "after-potential," has been most studied. In contrast with the spike it is very variable both as to magnitude and duration. It practically disappears in cold frog nerve and is inconspicuous in mammalian nerve unless exaggerated by laboratory procedures. In a fresh frog nerve it may last for a period as short as 20σ. After a period of stimulation its duration is increased, and when augmented by veratrine poisoning it is to be measured in seconds rather than in sigmas. The after-potential can be brought into existence only following a spike, and it was first thought to be a long drawn-out continuation of the latter; a number of observations, however, show that it is better to consider it as being caused by a separate process in the cycle of events which occurs in connection with a nerve impulse. In the first place the action-potential does not change form as a whole when a nerve is cooled; the spike is prolonged while the after-potential becomes shorter. The constancy of the spike and the variability of the after-potential have already

been mentioned; as would be expected from this observation, the two may be differentially modified by changes in the environment of the fibers. Certain substances which in the necessary dosage have but relatively little tendency to modify the spike have the power of greatly prolonging and augmenting the after-potential. Chief among this group is veratrine and to a lesser extent its pharmacological allies, protoveratrine and aconitine [10]. An analogous effect is also produced by an excess of the ions of the alkaline earths (Graham), Ba^{++} is the most effective, then follow Ca^{++} and Mg^{++}, while strontium falls a long way behind. In sufficient dosage all the foregoing substances can depress the spike, but this is not necessary for the effect on the after-potential to appear. Certain other substances which have a tendency to depress the spike have a much greater tendency to depress the after-potential. These are the univalent cations, particularly K^+, Rb^+, and NH_4^+ [9]; and the aliphatic narcotics. Asphyxia acts in a similar manner[1]. The anions are without effect (unless they are Ca^{++} precipitants) as one would expect from the general lack of permeability of the nerve for anions.

More important than the foregoing evidence in the differentiation of the after-potential from the spike is the fact that the after-potential does not always start out at a maximum. If the after-potential were merely a retardation in the decline of the spike, then whatever point we might select as the start of the after-potential should be at greater negativity than any subsequent point; but in numerous records this condition is not fulfilled, the after-potential shows clear signs of having a rising phase of its own. The best way to prepare a nerve for demonstration of a rising phase of the after-potential is to poison it with calcium or veratrine and then stimulate it rapidly. A response evoked in the period after stimulation may then show an after-potential which increases for 50 σ or longer. It is one of the maxims of pharmacology that drug action is unable to create any new processes in cells; all that it can do is to make a quantitative change in existing ones. When this change is an increase, pharmacology may be of great service to physiology because it may bring to light properties which would otherwise be missed. This was the case in connection with the rising phase of the after-potential; when it had once been found in poisoned nerve it was possible to demonstrate it in unpoisoned nerve. In this the usually bothersome diphasic artifact proved a help. It had been tacitly supposed that all the potential after the diphasic artifact was after-potential, but a close scrutiny of the diphasic notch revealed that in it there was a small deviation which was not accounted for (fig. 4, *B*). The explanation of this deviation was derived from a theoretical reconstruction of the diphasic artifact. For this purpose the most perfectly monophasic spike-curve available was employed. The second phase was placed in a position determined by the conduction time between a pair of leading-off electrodes, assigned a magnitude which would lead to the imitation of the potential form as recorded in actual experiments, and the two phases added algebraically. The summation curve (fig. 4, *A*) led to the hitherto unsuspected result that there is a remnant of negative spike-potential after the diphasic notch. This was labeled the T wave in analogy with a similar event in the electrocardiogram. Its usefulness lies in the fact that when its crest is once identified we know that from that time onward the spike potential will fall. If now the combined potential is still rising we have definite assurance that the after-potential must be rising, because the correction for the spike is a subtraction and not an addition. In many nerves the crest of the after-potential cannot be identified, probably because the crest is low and early and obscured by the end of the spike.

The significance of the foregoing detailed analysis lies in the fact that there are at one and the same time signs that one potential is rising while another is falling.

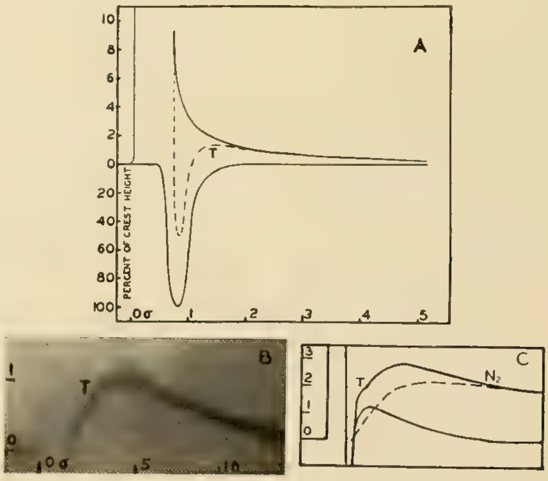

Figure 4. A reconstruction from a monophasic spike of a diphasic artifact having a potential 10 per cent. of the first phase.

B. Record of after-potential in a green frog nerve, 22°C., 3 mm. conduction, submaximal response.

C. Shows composition of *B*. Curve from *A* summed with theoretical form of after-potential to give the wave form recorded. All ordinates percentage of crest height.

Positive after-potential. The final restoration of the resting potential to normal occurs from the positive side (fig. 3, *C*). In a fresh nerve, in which the negative after-potential might last 20σ, the positive swing would be visible for about 200σ; under other conditions the period of positivity may be very much longer and after a tetanus of a minute, it may last a half hour. It is the only one of the nerve potentials which has a duration in any way comparable to the period of increased heat production after a tetanus.

Considerable doubt has existed in the minds of many physiologists, including myself, as to whether the positive wave represents any real process in nerve, because of the possibility that it may be an artifact occasioned by negativity at the dead-live junction; but a recent re-evaluation of the subject has convinced me that one cannot reasonably reject the obvious interpretation that the potential is due to a change in the positive direction under the active lead.

One of the assumptions on which the possibility of its being an artifact was based was that the negative after-potential is prolonged in the region of the junction with dead nerve, just as it is in a nerve which has stood for some time or has been much stimulated. It would then outlast the negativity at the active lead and record as an artifact in the positive direction. An actual lead made in the vicinity of the junction showed, however, that on the contrary the after-potential is small and short just as it is in very deteriorated or potassium poisoned nerve. Another explanation of the positive wave which has been proposed is that it is a temporary increase of the demarcation potential occasioned by a breakdown, as the result of activity, of a partially reformed plasma membrane at the end of the nerve. If this were the case the spikes recorded in the usual way should show a progressive decrease in their diphasic artifacts during a prolonged tetanus; but tests of this point show that the decrease does not occur. Finally, if a lead be made under the most perfect monophasic conditions, such as are obtained by the utilization of potassium for the production of the indifferent lead or of Bishop's cocaine method, the positive potential remains unabated, although the spikes do not show a trace of a diphasic artifact.

These are the potentials as they are known to occur in nerve. The spike-potential is the undoubted sign of the nerve impulse itself; it has never been found to be absent when the end effect has shown that an impulse has passed over a nerve. The after-potentials are very probably connected with restoration processes or the maintenance of nerve in the proper state to conduct spikes. This interpretation follows from their long duration, the necessity of a continued supply of available oxygen for the existence of at least the negative after-potential[1, 12], and the greater metabolism per spike which occurs when the after-potentials are large[12]. Further progress in the interpretation of the after-potentials must come through their correlation with other signs of activity.

For the theorist who would explain the origin of nerve potentials, the after-potentials have greatly complicated the problem, for three potentials now await elucidation instead of one.

Nerve potentials and the membrane hypothesis. The starting point of all theories is the membrane hypothesis, which postulates a polarized surface at the boundary of the axon capable of undergoing transient depolarization during activity. In its simple form the hypothesis is hardly more than a restatement of the facts and as such, affords a very convenient form in which to express the course of neural events. Historically the theory has been of much importance, but it has reached a stage in which there is danger of its becoming a means of lulling the mind into a state of satisfaction rather than of serving the proper function of a theory: the suggestion of experiments. Also, one encounters many statements made under the egis of the hypothesis which will not withstand analysis.

When a polarizing current is applied to a nerve, cations are carried to the inside of the membrane in the region of the cathode, and anions to the outside, in such a way as to oppose the polarized ionic distribution believed normally to obtain there (fig. 1). The implication is often encountered that this decrease of depolarization is directly responsible for the depolarization during activity; but a review of the subthreshold phenomena easily shows that such an interpretation is quite inadequate. The ionic migration produced by a just subthreshold shock must be nearly as great as it is at threshold, yet there is no trace locally of any effect resembling the spike. The depolarization which occurs during the spike must therefore be due to a process inaugurated by ionic movement and not to be due to the movement itself. It is of much greater magnitude than the preparatory depolarization and amounts more nearly to a complete disintegration of the surface.

What we need to know about nerve, more than anything else, is the immediate cause of the sudden local breakdown in the surface film. This perplexing point is usually glossed over in theoretical treatments by making the jump directly from the ionic concentration of the Nernst hypothesis to the depolarization idea of the membrane hypothesis, although Bernstein, in his pioneering formulation of the subject, proposed in no uncertain terms the intervention of a chemical link at this point. The importance of this stage in the

cycle of activity is illustrated by the beauty of the mechanism. For short periods at least, frog nerve at room temperature may produce 800 spikes per second; in theory this means 800 depolarizations per second and as many restorations (the depolarizations are, of course, much smaller than normal at this frequency).

The language of the membrane hypothesis treats the membrane as though it had a separate existence. This is justifiable only on the ground that the language is figurative. It is well to keep in mind that the barrier at the surface of a cell is maintained only at the cost of a constant expenditure of energy and that it disintegrates if the cell be deprived of oxygen for any length of time. The composition of the cell-surface must vary with the cellular metabolism; that is, with the molecular species available at the moment for concentration in the surface, according to the Gibbs-Thomson principle. Conversely, if the surface be altered by external means it is no longer in equilibrium with the interior, and one would expect the metabolism to change. In other words, there is a continuous reciprocal relationship between the surface and the cytoplasm. The surface changes may well be attended by potential changes, and by some such mechanism as this the after-potentials may be accounted for. Whether the spike, with its larger potential and different properties, can be accounted for on the same basis is more uncertain. In any case the idea that the surface potential is a reflection of several processes in a nerve makes it understandable that one potential may be rising while another is falling.

The size of the spike has always been associated with the magnitude of the demarcation potential. If the propagated disturbance be a depolarization phenomenon, the height of the spike is determined by the difference between the resting potential and that at the point of maximum depolarization, from which it follows that the spike height could theoretically never be higher than the demarcation potential. This condition seems to have been satisfactorily met by experiment, but only after special precautions to obtain the demarcation potential at full value. In turn the demarcation potential is best explained on the basis of a concentration difference on the two sides of the plasma membrane. The idea was originally launched by Bernstein, with the proof that the potential is determined at the intact surface and that its size varies with the absolute temperature; and the subsequent history has been such as to support the notion. The concentration difference between the potassium inside and outside the fiber has been found to be sufficiently great to produce the potential (Cowan), and physical chemistry has provided two very satisfactory models which show ways in which a concentration potential can be exerted without the presence of metals.

One consequence of the concentration-cell theory is of special interest. If increased permeability, and therefore low resistance, be characteristic of an active region, current must flow through the active region from the inactive ones. When a concentration cell supplies current it gets the energy from its surroundings, therefore the nerve should tend to cool just as does an electric organ—particularly if the organ is caused to discharge through an external resistance in which the energy can be expended(2). If, now, the energy of the local bioelectric currents be again all dissipated as heat, the net effect on a thermopile placed on the outside of a nerve should be zero. In an actual experiment, however, a thermopile records some initial heat. On account of the theoretical importance of the question of the existence of initial heat, A. V. Hill has examined his thermal data to see whether they are susceptible of explanation on the basis that all the heat produced is recovery heat. He has come to the conclusion that a certain small portion of the heat must be considered as produced at the time of the impulse. This means that there must be some additional exothermic process; and it is quite possible that this process may enter into the mechanism at the stage in which the effects of ionic accumulation lead to the final opening of the membrane.

While we speak of the spike as due to a depolarization of the plasma membrane, it would be quite incorrect to think of it as a complete depolarization. This follows from the behavior of the spike in cooled nerve. While the demarcation potential falls off as the absolute temperature the spike height falls off very sharply and at the same time becomes much longer. Thus the spread between the resting and activity potentials becomes narrower. To account for the change we may make use of a possible increase in viscosity in the surface film, but we must also consider the effect of temperature on the final unknown event responsible for the depolarization; if that were understood we would be much farther on our way to the understanding of nerve.

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Discussion

Dr. Irwin: I think that the temperature effect on the spike potential may be a matter of viscosity. The temperature coefficient of viscosity in chloroform is very large.

Dr. Abramson: Would that be a change in the sheath, something analogous to gel formation, or to an increase in the Newtonian coefficient of viscosity?

Dr. Gasser: Viscosity and perhaps conductivity. The temperature coefficients of viscosity of some of the oils are very much like those of the nerve functions. Also specific conductance falls off very rapidly when a lipoid approaches its congealing point, as for instance tetraethylammoniumbromide in a mixture of alcohol and lecithin.

Dr. Abramson: It has to do primarily with the true viscosity, then, for there is very little change in the electrolytic conductance during the sol-gel transformation.

Dr. Blinks: Was it a single phase lipoid or an emulsion?

Dr. Gasser: Probably an emulsion.

Dr. Cole: As I remember Hill's calculations, the energy of the initial heat would not be far from what you might compute the total electrostatic energy stored in a membrane to be, considering it as being a condenser.

Dr. Gasser: That is correct. Hill has also proposed the cycle; discharge of the condenser and recharge by means of a diffusion potential, with a net heat for the cycle of zero.

Dr. Cohen: The single nerve fibers vary, of course, in diameter. Do the action potentials show any relation to the diameter?

Dr. Gasser: Yes, the spikes are longer in small fibers, but the big medullated nerves have spikes that are very much alike in their duration. The fact that the latter have velocities ranging from 30 to 10 meters a second is not due to the duration of the spikes but must be connected with the size of the fibers—the variable which makes the difference in velocity is the diameter of the fiber not the cross section. The relationship is a practically linear one.

Dr. Abramson: Can you reverse the demarcation current in nerve?

Dr. Gasser: G. H. Bishop has shown that treatment of a portion of a nerve with cocaine or one of the aliphatic narcotics causes that region to become slightly positive to an untreated portion. But if the nerve be cut across the demarcation, current is always from the outside through the cut end to the inside.

Dr. Blinks: When you suppress the after-potential what effect on heat production is observed? Would it tend to throw the recovery process closer into the spike or inhibit its recovery?

Dr. Gasser: This subject has not been studied directly. As it is the opposite state from that obtaining in the veratrine experiment, one would expect the oxygen consumption and heat production to be small. The interpretation might be absence of the normal restitution, but other interpretations are possible.

END OF COLD SPRING HARBOR SECTION

MORPHOLOGICAL AND ELECTROPHORETIC EFFECTS OF THE GALVANIC CURRENT ON GRIFFITHSIA CELLS

Victor Schechter

Instructor in Biology, College of the City of New York

Electrical energy in its application to living systems is of peculiar interest in that, unlike most other forms of energy, organisms are not ordinarily subjected to it in nature. Yet, they are capable of response and, furthermore, they themselves produce electricity in amounts ranging from the distinctly perceptible voltage of the electric fish down to quantities so small that they are measurable only with uncertainty. The latter magnitudes are, of course, the usual ones and some workers have attached large significance to the fact that in no case where measurement is possible has the maintenance of potential difference been found to be absent.

I wish to report here tonight, in abstract, some work with the red alga *Griffithsia bornetiana*, a form which I have found especially suitable for electrical experiments.

The morphological polarity of *Griffithsia* is established very early. After many nuclear divisions the cell divides once to form the rhizoid, and again to form the shoot. This takes place in a day or two. By continued rapid division the thallus is formed. Here we have branching chains of cells ranging in size from about 60 micra at the apex of the plant to 2000 or more micra at the base. The basal cells are attached by means of rhizoids.

For the purpose of the experiments fragments of a thallus were placed in the experimental apparatus and oriented alternately with and against the current. The central part of the apparatus consists of a rectangular glass dish with sloping bottom to provide a more or less uniformly graded current of known intensity. The rest of the attachments shown are for lighting, water exchange and the introduction of the current without products of electrolysis. After two or three days of electrical treatment a very interesting picture could be seen.

In fragments which were oriented with base toward the anode, the rhizoids originated at the base ends of the cells, that is, toward the anode, and the appearance of the completed thalli was more or less normal. However, if the fragments were oriented with the apex toward the anode, the rhizoids were again produced toward this pole and thus at the apical ends of the cells. We have here a morphological indication of reversal of polarity. With the proper current strength every rhizoid formed, and there may be as many as 40 or 50 in a fragment consisting of 80 to 100 cells, originates at the apical end of the generative cell when the apex is toward the anode; and in those fragments oriented the other way every rhizoid is in its natural position at the basal end of the cell.

Accompanying the gross morphological effect just described are correlated intracellular phenomena of unusual interest. First, there can usually be seen in the thallus as a whole a gradient of color ranging from a deep pink in the cells toward the cathode, much more intense than the usual hue of the cells, to a pale tan in the cells toward the anode,—a shade rarely found in untreated cells. This color effect is, of course, independent of the position of the fragment. A second intracellular effect is the aggregation of chromatophores and probably other cytoplasmic components toward the anode of each cell. It may be of significance in this connection that the first indication of rhizoid producton under normal conditions is the formation of a visible pigment concentration, which then pushes out as the cap protoplasm of the rhizoid .

To return to the color changes observed, it must be of some significance that rhizoid formation in inhibited in the basal region or completely stopped in the apical region when these, by virtue of their orientation, assume the pale tan color. A pH change may be involved. This is supported by the fact that in acid the cell pigment approximates the deep pink color observed toward the cathode of the electric field, whereas in alkali it becomes pale and finally greenish in hue. Other evidence indicates that the electrical effect on color is not a pH change in the most usual sense; that is, it is not due to the production of acid or alkali in the cells but rather to a migration of materials which themselves may produce relative acidity ar alkalinity by derived differences in concentration. This evidence was obtained by mounting the material in a miniature apparatus on the stage of a microscope.

With high currents each cell becomes deep pink toward the cathode and green toward the anode. If the current direction is now reversed, after a few minutes the colors reverse their position. Observations during the progress of this change show a fading of the pink at the anodal end before any pink color appears at the cathode. In this way there is a slow shift of pink intensity

from anode toward cathode, pointing to the probability that the effect is not a pH change at the cell membrane. In the latter case we would probably see the simultaneous appearance of pink and green at the respective cell membranes rather than a slow reversal of color.

The possible integration of these morphological and intracellular effects must be delayed pending further data. I wish only to point out at this time the co-existence of the described intracellular phoretic phenomena with the determinative action of the electric current during regeneration.

(This article is based upon a seminar presented at the Marine Biological Laboratory on August 10).

THE ABSORPTION OF COLLOIDAL CARBON BY THE MESONEPHRIC EPITHELIUM OF NECTURUS MACULOSUS

DR. ALDEN B. DAWSON
Associate Professor of Zoology, Harvard University

Colloidal carbon in the form of diluted Higgin's waterproof India ink, when injected intraperitoneally, reaches by way of the nephrostomes and peritoneal canals the lumina of the primary and secondary tubules of the mesonephros of *Necturus*. Usually it is absorbed and stored only by the brush-border cells of the proximal convoluted segment. When blockage of the distal segment occurs, the suspension is forced by the ciliary pressure of the peritoneal canal into the capsule; carbon is then also stored in the epithelium of the outer wall of the capsule.

The amount of carbon stored by brush-border cells is very variable and could not be correlated with either the amount or duration of the injections. Heavily laden epithelial cells may be desquamated, and the epithelium is repaired by thinning of the cells followed by multiplication by mitosis.

There is an extensive infiltration by carbon macrophages from the peritoneal cavity. These cells accumulate chiefly in the interstitial tissues but may infiltrate the renal epithelium and in many instances reach the lumina of the tubules and the capsular spaces.

Ink injected intravascularly was found not to reach the mesonephric epithelium. Even the endothelium of the peritubular capillaries had not taken up any carbon after a forty-eight hour interval although the reticulo-endothelial cells of both spleen and liver contained large amounts.

COLOR CHANGES IN THE DOGFISH

(*Continued from Page* 233)

represent the distribution areas on the skin of the nerves concerned. We therefore believe that the light phase of the dogfish is not due simply to the absence of pituitary secretions, but to the positive activity of the nerves.

We agree entirely with Lundstrom and Bard that the darkening of the dogfish is due to the pituitary secretion, but we attribute the light phase of the animal to positive nerve action. In this sense, the dogfish is the first fish to show light responses on the severance of its nerves. It presents an unusual condition in that its dark phase of amphibians, depending upon a hormone; whereas its light phase is caused by direct nervous action as in most other fishes.

(This article is based on an article presented at the Marine Biological Laboratory on August 8).

THE AUSTIN ORNITHOLOGICAL RESEARCH STATION

DR. O. L. AUSTIN, JR., *Director*

The Austin Ornithological Research Station at North Eastham, Cape Cod, while a protected sanctuary for all fauna whose presence is not seriously incompatible with bird life, is devoted to ornithological and closely related biological investigations. Although owned and financed privately it has a cooperative agreement with the U. S. Biological Survey.

The Station's six hundred acres on the bay side of the King's Highway, comprises land of all types found on the Cape, affording a great variety of ecological associations. Salt and fresh water marshes, an extensive fresh water pond, two salt ponds, woods of large pitch pines, scrub oak and locust thickets, open meadows, an asparagus field and even old burned land, each attract different avian species. Cultivated fields of buckwheat and millet and planted berry-bearing bushes afford winter bird food, while at all seasons grain of suitable sorts is broadcast in an amount sufficient to maintain all avian guests. Attention to their individual preferences in the matters of shelter, food and environment attracts for study all species found on the lower Cape, affording abundant material for specific investigations, as well as indicating how greatly alterations in environment affect bird populations.

An enlarged and remodelled old Cape Cod dwelling shelters in comfort the staff and its guests, houses the scientific records and an ornithological library of over four thousand volumes which is comprehensive in the bird literature of Eastern North America. A commodious barn provides a workshop for the construction of traps and affords storage room for grain and necessary gear of all sorts. Automobiles of different types and equipment facilitate access to any desired locality from a traverse of scrub woods to a long trip over sand beaches. In project is the construction of a small building for research work where laboratory facilities will be better than they are at present in the Station home.

Bird-work is carried on uninterruptedly the year round with always one, rarely less than two, scientifically trained and adequately experienced workers in residence. During migration and nesting seasons the staff is augmented so that at times six men are necessary for the work at hand. Aside from his part in the routine activities, each collaborator has at least one problem for investigation and conclusion.

Since the identification of individuals is essental to the study of free birds, banding has been adopted as the major undertaking. By means of baited traps varied to suit the species and seasons, and by Italian bird nets, birds are captured, ringed with numbered and suitably inscribed bands and liberated immediately. In three years over 43,000 individuals of 162 species have been identified thus, while the recapture of these has resulted in the handling of over 88,000. Many of these birds return to the free food in the traps so regularly that it is possible to determine their dates of arrival and departure and to some degree the extent of their local wanderings. The large number of captures elsewhere, even so far as in South America, of birds banded at the Station and the reverse of this, gives clues to the times and routes of migration. Elaborate records are kept of all captures, bandings and ornithological observations. Card systems hold complete individual histories of "repeaters." Thus there is being accumulated an enormous amount of data of value in the consideration of any problem. As extensively as time and opportunity permit, motion picture film is made to record not only anatomical characteristics but more especially behavior.

Aside from the routine basic work already mentioned, material is being collected which will result in a comprehensive description of the Cape's avifauna. This summer endeavor has been concentrated on the several large tern colonies. Last winter photoperiodism was studied with ducks and white-throated sparrows. The more academic biological investigations under way embrace botulism, communism in terns, avian malaria and the etiology of the varying seasonal survival rates of Tree Swallows.

The Station has no museum and no caged living birds to entertain visitors. To laymen who drop in, the work on hand at the moment is described and illustrated in the field to a degree considered adequate to the stimulation of popular interest in ornithology and conservation. To ornithologists and zoologists, the Station's records, field material and assistance are available at all times.

The School of Science held its Semi-Annual Meeting on Friday afternoon, August 18 at half past two. It decided that hereafter no child under seven could be permitted to attend the classes as it requires teachers especially trained in the handling of small children. The treasury is not in a position where it could finance such an undertaking.

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Jean M. Clark, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

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THE PUBLICATION OF THE SEMINAR REPORTS OF THE MARINE BIOLOGICAL LABORATORY, I.

The Marine Biological Laboratory has announced its plan of publishing abstracts of the papers presented at the evening meetings in the October number of *The Biological Bulletin.* Recently the following unsigned memorandum was sent out to those persons scheduled to take part in the seminars:

"The Biological Bulletin will publish in the October issue the titles appearing on the program of the evening scientific meetings and the general meeting to be held in the latter part of August. It will also publish abstracts of the papers presented, at the option of the authors. The Biological Bulletin does not wish to publish abstracts based on material which has already been published or which will be published before the first of November in other scientific journals, but will gladly record the place of such publication along with the title of the communication.

"Abstracts should be as brief as possible, and should in general not exceed three hundred words, including its equivalent in tabular matter. When circumstances warrant it, abstracts not exceeding five hundred words will be accepted. No illustrations will be published. Abstracts should be submitted to the Biological Bulletin, Room 305, prior to the time when the paper is presented."

Certain investigators have interpreted this note from the Director's office to mean that prior publication of seminar reports in The Collecting Net would exclude abstracts of them in *The Biological Bulletin.* In view of this misunderstanding it seems worthwhile to quote part of a letter received from the editor of the latter publication who writes under the date of July 5: "we do not feel that this policy[1] should interfere with your dealing with these papers in The Collecting Net as you have in the past." Further he wrote that we might assure investigators that publication of their seminar reports in The Collecting Net would not prevent their later publication in *The Biological Bulletin.*

[1] That of publication in "The Biological Bulletin."

Introducing

Dr. John H. Northrop, who was born in Yonkers, New York in 1891. He was graduated from Columbia in 1912 with degrees of B. S. and received the degree of M. A. in 1913 and Ph. D. in chemistry in 1915. He attended the course in Invertebrate Embryology in Woods Hole in 1910. He was appointed to the Cutting Travelling Fellowship and worked with Dr. Jacques Loeb at the Rockefeller Institute. He was appointed to the staff of the Rockefeller Institute in 1917 and part of that summer and the following one were spent in Dr. Loeb's old laboratory in Woods Hole. He continued his work in the laboratory of the Rockefeller Institute and was made a member of the Institute in 1924. In 1926 he moved his laboratory to the Princeton laboratories of the Rockefeller Institute. Two years ago the Stevens prize of the College of Physicians and Surgeons of Columbia University was awarded to Dr. Northrop.

He has been interested primarily in physical chemistry of proteins and enzymes with occasional work on the duration of life with *Drosophila* and the study of bacteriophage. He is one of the editors of the *Journal of General Physiology.*

Dr. Northrop is spending the summer in West Falmouth with his wife and two children, and is working at the Marine Biological Laboratory.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 23	6:23	6:40
August 24	7:02	7:24
August 25	7:43	8:11
August 26	8:28	9:02
August 27	9:17	9:56
August 28	10:12	10:57
August 29	11:13	....

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

ITEMS OF INTEREST

On the evening of August 21, a bust of Mathew Fontaine Maury was presented to the Woods Hole Oceanographic Institution by Mr. Charles R. Crane. Dr. Frank R. Lillie, president of the Corporation, accepted the bust for the Institution. Mr. Crane gave a brief address, telling how he first became interested in the pioneer oceanographer and outlined the many things which Maury had done to establish the ground work upon which rests the present research work of the Oceanographic Institution. The dedicatory exercises were held in the chart room on the second floor where the bust now stands upon the mantel piece above the fireplace. The audience was a carefully selected one, consisting of the trustees and a few especially invited guests. Refreshments were served in the chart room which was attractively decorated with flowers.

Dr. David H. Tennent, professor of biology at Bryn Mawr, his wife and his son, David, who will be a sophomore at Yale next year, are spending a month here in the small Danchakoff cottage.

Mrs. Jaques Loeb, her son, Dr. Robert F. Loeb of the College of Physicians and Surgeons, Columbia University, her daughter, Mrs. Edward B. Osborne and her two grandchildren are spending the summer at Woods Hole. Dr. Edward B. Osborne spends the week-ends with them.

Dr. Philip Bard has been appointed professor of physiology at the Johns Hopkins University School of Medicine.

The motion pictures of tissue culture technique which will be shown in the auditorium on Thursday were prepared by Dr. Robert Chambers and Mr. C. G. Grand for exhibition at the Chicago World's Fair. The pictures have been sponsored by the Society for the Control of Cancer, in order to acquaint the laymen with certain aspects of the cancer problem.

Dr. Reid Hunt, professor of pharmacology at the Harvard University Medical School is visiting Woods Hole.

Among those who have recently completed their work at Woods Hole for the summer are: Dr. and Mrs. Krogh, Dr. Coghill and his daughter, Dr. Schotté, and Dr. Gerard.

Dr. S. A. Waksman recently received a letter from Hanover, Germany, which was opened by the censor before it left the country. It was resealed with a sticker bearing the words, "Zur Devisenüberwachung Zollamtlich geoffnet."

Professor Kenneth S. Rice, associate professor of biology at the University of Maine, is visiting Woods Hole with his family. They are living in the dormitory until their rented house in the Gansett Woods will be vacated early in September.

Dr. A. M. Reese, professor of zoology at West Virginia University, has written that he will not visit Woods Hole this summer. An article by him on the treatment of snake venom will appear in an early number of *American Medicine*.

Julian P. Scott has returned to Woods Hole after an extended period of travel and is holding an exhibition of photographs of Woods Hole personalities and other scientists at the Old Lecture Hall. Mr. Scott took a number of pictures of scientists at the recent International Entomological Congress held at Paris. Negatives of all the pictures in the collection are with the W. F. Roberts Co., 829 17th Street, Washington, D. C.

The contributor of the two articles on "Impetigo" and "Epidermophytosis" were written for The Collecting Net by Dr. Austin W. Cheever of the department of dermatology at the Harvard Medical School. In error, the articles were credited to another Dr. Cheever at the same institution. The author's father recently spent two summers at Woods Hole.

Owing to the note which we printed recently concerning Dr. Elbert C. Cole's book, "An Introduction to Biology," the author has written us a note correcting our statement by saying that this volume is intended primarily for high school students and the entire treatment of the subject was simplified as much as possible in order to bring it within the range of high school boys and girls.

On Sunday evening, August 27th, there will be a concert by the Washington String Quartet for the benefit of the Woods Hole Public Library in the M. B. L. auditorium. Tickets at fifty cents and one dollar are on sale by Miss Polly Crowell at the M. B. L. office and at the Woods Hole Library.

THE COLLECTING NET PRESENTS WHALING FILM FOR THE BENEFIT OF ITS SCHOLARSHIP FUND

For those whose imaginations are stirred by brave tales of the sea and the men who follow it, a treat has been prepared by The Collecting Net. On Monday evening, Aug. 28th, Mr. Chester Scott Howland, lecturer and son of an old New Bedford whaling captain, will give a lecture "Hunting Whales in the Seven Seas," illustrated by moving pictures of his own making, for the benefit of The Collecting Net Scholarship Fund in the Marine Biological Laboratory. The theme concerns those whalers of New Bedford and Nantucket whose vessels sailed the seven seas in search of fortune, the "praying deacons" who left their Cape Cod plowshares at the age of fourteen to answer the call of the sea, of rigging and harpoons, of the toll of the sea, and the lore of whalers and whaling ways. The pictures are extremely interesting and show the methods of whaling before the romance of the windjammer gave way to the progress of steel, and the whale-oil lamp to the incandescent bulb.

Mr. Howland gave a similar illustrated lecture, entitled "The Tale of an Ancient Mariner" for the benefit of the Scholarship Fund six years ago. At that time he was introduced by Dr. E. G. Conklin, who said he would give ten years of the peaceful years of his life to repeat the experience of being towed by a harpooned whale. In speaking of that lecture Dr. Conklin said: "The audience was one of the largest ever assembled in our new auditorium—and safe to say as critical as you will ever address. The lecture was both interesting and profitable. I have heard only words of praise for your presentation of the subject."

The lecture Mr. Howland will give on August 28th is an improved version of the old one, with a number of new reels of motion pictures and much new and interesting material added. Mr. Howland is a son of Capt. George L. Howland, for many years the skipper of the bark *Canton*. In 1890 Captain Howland was honored by the government of Great Britain for the heroic rescue of sixteen members of the crew of the bark *British Monarch* burned at sea 700 miles off the coast of Africa.

Among those warmly endorsing Mr. Howland's lectures, in addition to Dr. Conklin, are Professor Thomas N. Carver of Harvard University, Professor Robert Chambers of New York University, George U. Denny of Columbia University, Professor Stanley E. Ball, Curator of the Peabody Museum, Yale University, and William F. Macy, president of the Nantucket Historical Association.

THE CONGRESS FOR EXPERIMENTAL CYTOLOGY

The Third International Congress for Experimental Cytology will convene at the beginning of next week in Cambridge. Among those persons who have recently been at Woods Hole, the following will take part in its sessions: Dr. Robert Chambers, "Some features of cell permeability in relation to kidney function"; Dr. W. J. V. Osterhout, "The Electro-physiology of vegetable cells"; Dr. L. Michaelis, "The reduction intensity of the living cell"; Dr. R. Beutner, "The vital battery system"; Dr. S. C. Brooks, "The relation between ions and potential differences across plasma membranes"; Dr. S. Gelfan, "The degree of independence between the contractile and conductile mechanism in the muscle fibre"; Dr. C. Speidel, "Growth and repair of nerves."

The program of the Congress is divided up into several all day symposia. Dr. E. Fauré-Fremiet, who recently spent a summer at Woods Hole, is chairman of the symposium on "Cell form and function as demonstrated by recent advances in tissue culture." Dr. W. J. V. Osterhout leads the symposium on "The Electro-physiology of vegetable cells."

THE JAPANESE BEETLE

During the summer at different times people have brought to us insects to be named, which they thought, or feared, might be the Japanese Beetle. So far, these have proved to be other forms of insects.

We have felt, however, that it would be well to have a set of these beetles so that any one who desired to see them could call at the office of the Supply Department, or at the Museum, where someone would willingly show the set consisting of larva, pupa, and the adult male and female beetles.

The beetles themselves are quite attractive, having a shiny copper-bronze colored body, and green head.

The State is quarantined against these insects, but we would advise anyone finding the Japanese Beetle to notify Mr. Frank Bartley of Falmouth Heights, who is a landscape gardener and tree warden; as we feel that all steps should be taken to eradicate this pest, if possible.

George M. Gray,
Curator of the Museum.

There will be an informal exhibition of sculpture at the Breakwater Hotel showing the work of Bryant and Robert Baker. Among the busts that will be shown are several of people from Woods Hole. The exhibition will be open August 26, 27 and 28.

THE COLLECTING NET

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THE DIFFERENTIATION OF THE PROTOPLASM OF EGG CELLS DURING EARLY DEVELOPMENT

Dr. Joseph Spek
Professor of Zoology, University of Heidelberg

A long series of experiments has been done on various types of egg cells in order to study the origin of that organization of these egg cells which controls their further development. Many of these types of egg cells have a more or less concentric organization before the formation of the polar bodies. Below the surface there is an accumulation of specific substances which sometimes form a sharply separated "cortical layer." A careful study shows that in the eggs of some animals this cortical layer itself consists of several components arranged in a regular manner in several concentric layers. In the center of the eggs the large nucleus is situated: around the nucleus, regularly distributed, are the microscopical components of the yolk, the different kinds of granules and droplets. The concentric arrangement of the components of these egg cells is transformed after (*Continued on Page* 300)

WAVE TRANSMISSION AS THE BASIS OF NERVE ACTIVITY

Dr. A. V. Hill, F.R.S.
Foulerton Professor of the Royal Society

Wherever we look in the world of matter and events outside ourselves we find that oscillations and wave motion have a significant, often a dominant, rôle. It is not, therefore, astonishing to find that waves play an important part in ourselves also. Let us discuss the nature of these waves.

Most of the well-known oscillations with which physics is concerned are a consequence of the reaction with one another of properties analogous to inertia and elasticity. A moving or a changing system tends, on the one hand, to continue in its state of motion because it possesses, for example, mass or inductance: even social, economic and intellectual changes are endowed with such characters of inertia, which keep them going when they have passed a true position of equilibrium. On the other hand, such systems, if they are to continue to exist, if they are not merely to be dissipated, must possess converse properties which

M. B. L. Calendar

TUESDAY, AUG. 29, 8:00 P. M.

Seminar: Charles B. Wilson: "The Copepod plankton."

D. E. S. Brown: "The pressure coefficient of viscosity in the eggs of Arbacia."

Edwin P. Laug: "Observations on lactic acid, total CO_2, and pH of venous blood."

Anna R. Whiting: "A study of eye color in the parasitic wasp, Habrobracon."

Marc A. Graubard: "The melanin reaction in races of Drosophila melanogaster."

THURSDAY, AUGUST 31st

General Scientific Meeting. (See page 301).

FRIDAY, SEPT. 1, 8:00 P. M.

Lecture: Prof. Robert K. Cannan: "Studies in the amphoteric properties of proteins."

TABLE OF CONTENTS

A PHOTOGRAPH OF THE WORKERS OF THE MARINE BIOLOGICAL LABORATORY TAKEN JUST FORTY YEARS AGO

Front row, left to right: S. Watase, F. P. Gorham, N. R. Harrington, Ira van Giesen, J. E. Peabody, F. S. Conant, E. G. Gardiner, W. T. Councilman. *Second row*: H. G. Dyar, F. R. Lillie, F. J. Brockway, J. P. McMurrich, not identified, H. Randolph, K. Foot, C. O. Whitman, H. B. Merrill. *Third row*: W. L. Poteat, G. N. Calkins, A. P. Mathews, W. H. Dudley, (?), E. G. Conklin, W. H. Everett, not identified, C. W. Dodge, A. D. Morrill, F. S. Lee, not identified, E. E. Bickford, C. Langenbeck, E. F. Byrnes. *Fourth row*: A. L. Treadwell, J. Loeb, T. H. Morgan, H. Ayres, J. B. Platt, not identified, E. T. Brewster. *Second and third window*: P. A. Fish, W. M. Rankin, A. D. Mead, H. C. Bumpus, G. Gray.

tend to bring them back once they have overshot their equilibrium: such properties in physics are elasticity and electrical capacity, in finance and politics are fear and conservatism. These exercise a constraining force increasing with the displacement from equilibrium, and ultimately reverse the motion or change, and the same oscillation is repeated in the opposite direction.

There is, however, another type of oscillation, less commonly discussed in physics or mechanics but none the less well known in every-day affairs; that which depends upon a discharge taking place when some limiting potential or intensity is reached. For example, (1) water falling into a tank equipped with a siphon will come out in rushes whenever it rises to a certain level. Or again, (2) a population in which an epidemic of measles can not start because of the number of people in it who are immune, having had the disease already, will gradually become less immune as time goes on, and finally an epidemic, a wave, of measles will sweep through it. (3) A neon lamp with a parallel condenser, in series with a resistance and a source of electromotive force, will discharge at regular intervals, namely, whenever the potential difference across the condenser reaches a certain critical value. This type of oscillation does not depend upon inertia reacting with elasticity. Its essential nature is (a) that some state, some potential, some intensity, is built up by a continuous process, and the condition becomes less and less stable until one is reached in which discharge must take place, and (b) this discharge, once started, forms a path for itself by which (as in a siphon or an electric arc) further discharge is facilitated until what has been built up gradually has been broken down and the process begins again. This type of oscillator (sometimes referred to as a relaxation oscillator) is the one with which alone we are concerned in physiology.

Waves may be transmitted on the same principle in a system extended in space. An unstable state is gradually built up until at some point, either through an external agency or by some intrinsic process, discharge is begun, which starts and facilitates a discharge in neighboring regions which themselves discharge, and so a wave is propagated. Such waves will occur periodically if at some region the potential at which discharge begins is less than that finally attained by the continuous process of charging: they will require, however, an external agency (a "stimulus") to start them if the unstable condition, the limiting potential, is not attained spontaneously. Models of such waves will occur to all of you: their principle is obvious. I have emphasized it because the waves on which nervous activity is based appear to be of this type. All detailed theories of nervous transmission may well be wrong, but this general idea of it, involving building up and discharge, is almost certainly right.

One of the chief characteristics of living systems, particularly of animal ones, is "excitability" —the property of reacting to a small change in the environment, or even in the system itself, by a greater response. This excitability is zero immediately after a response has occurred: the cell or organ passes through a completely refractory stage: its potential is at first not yet high enough for any further discharge to take place. After the completely refractory phase, it passes into one in which its excitability is lower tnan normal, in which stronger provocation than usual is required to produce a discharge. Finally it returns to its initial state. This state may be one in which spontaneous discharge occurs, or, on the other hand, discharge may require what is known as a stimulus to start a path for it. In a single organ the final level may be above or below that of spontaneous instability, according to the circumstances. The greater the constraint on it the more quickly, in general, it reaches the level of discharge, the greater the frequency of the so-called "response." The time for the complete cycle from the absolute refractoriness following a response to complete recovery ready for further discharge varies greatly from one organ or cell to another. It may be measured in thousandths or in fractions of a thousand or a second, it may be measured in seconds or even minutes.

An ordinary nerve trunk is a bundle of separate fibers along which messages are sent. The unit, the nerve fiber, is not normally spontaneously unstable, but it can be excited by various agencies, particularly by an electric shock, after which a wave starts off from the point excited and travels to the end of the nerve. Once the discharge has been started it persists and travels in space. If a nerve be injured persistent spontaneous discharge may occur. An ordinary muscle, which is a bundle of separate fibers which contract and do mechanical work, is not spontaneously excitable, though it also can be stimulated by various agencies; it is easy, however, by a slight change in the salt content of its environment to render its state unstable, so that regular oscillatory discharges take place in it; these can be recorded electrically and result in visible twitchings of the fibers. The heart muscle, on the other hand, has naturally an inherent rhythm of its own; after a beat it is at first completely refractory, then for a period it can be excited, *e.g.*, by an electric shock, finally it beats again of itself. Something in it is gradually restored, until a potential is reached at which discharge must occur. This restoration is quicker in one region than in another, and since the discharge once started is

propagated as a wave, the rhythm of the heart as a whole is set by that of the region in which restoration is quickest. All parts of it, however, have the same property of spontaneous discharge, and even an isolated portion of heart muscle will beat of itself, though more slowly than when attached to its natural pace-maker. If its mechanical tension be diminished enough the spontaneous beats may disappear; the lack of mechanical constraint somehow raises the level at which instability is reached and spontaneous discharge occurs.

It is necessary sometimes for a physiologist, as Rutherford was once heard to say, to make a noise like a physicist. Physics is not indeed the only, or even the chief, way of approaching physiological problems, but it has among its advantages that of providing at such a conference as this a language by which other people, for example, engineers, physicists and chemists, may be introduced to physiology. Through no fault of their own, no doubt, many people, people of high scientific standing, have never had any experience at all of that subject. They often have very absurd ideas about it. They do not perhaps go so far as the lady who, to a doctor trying to explain to her what was wrong with her, made the appeal, "Don't, Doctor, don't—I like to think I am lined with pink satin." But they are apt nevertheless to picture the inside of the body, if not as pink satin, at least as beyond the range of reasonable scientific method. If, as I hope, there are a few engineers, physicists and chemists here, I would reassure them: physiology, and in particular that of the nervous system, is an experimental science like any other: no doubt it requires great experimental skill, but that makes it the more amusing: it is complex, but not beyond the wit of man to investigate: its complexity depends partly upon the difficult nature of its experimental unit, the single living cell, partly upon the fact that most of the effects which can be observed are due to the action and interaction of very many of these units.

The nerve fiber in which the waves run is part of a nerve cell: the central part of the nerve cell usually lies in or near the nervous system: the fiber—the axon—runs out to the organ, muscle, gland or sensory ending, with which it is connected. The fiber is a fine thread of protoplasm, a few thousandths to a few hundredths of a millimeter in diameter but often of considerable length. The velocity with which waves are propagated in these fibers may be anything from 100 meters to a few centimeters per second. In our own motor or sensory nerves the speed is somewhere near the upper of these limits.

The messages which pass in nerve fibers can be detected by various means apart from the response, *e.g.*, movement or sensation—which they provoke. The chief of these depends upon the fact that each impulse has an electrical accompaniment, which, owing to recent improvements in electrical technique, can be easily and accurately recorded. Not only, indeed, can we see nerve waves chasing each other along the screen of an oscillograph, not only can single impulses in single fibers be recorded photographically, but we can even listen on a loud speaker to sensory impulses caused, for example, by gentle pressure on the toe of a cat. As Adrian says, there would be no particular difficulty in demonstrating the potential change in a frog's nerve fiber as an audible signal on the radio. The power in the input circuit would be of the order of 10^{-14} watt, the transmitter might radiate 50 kilowatts, five million million million times as much. It would, as he adds, be a sad confession of failure if with these resources we had learned nothing fresh about the working of the nervous system. As a matter of fact, we have.

In, and in connection with, the living body itself as part of the complex telephone arrangements of the central nervous system, this electrical sign of nerve activity is the chief means by which in the last few years the subject has grown so fast. Other signs, however, there are, and the investigation of these has led to considerable advance in knowledge of the physical nature of the nerve wave itself. For example, when a single impulse travels down a medullated nerve there is an immediate rise of temperature of the order of 10^{-7}° C. (one ten millionth of a degree). This represents a liberation of energy, small, indeed, but perfectly definite, of about 4 ergs per gram of nerve. A single fiber one hundredth of a millimeter in diameter, if it weighed one gram, would need to stretch about 10 kilometers. Thus, in sending a single nerve impulse 10 kilometers, the amount of energy immediately set free would be about 4 ergs. One gram-calorie would send it 10^{8} kilometers, about half the distance to the sun. Clearly, communication by nerve fiber is not very expensive!

Let us remember, however, the number and variety of nerve waves involved even in ordinary activity. Nothing perhaps can better illustrate nervous action than a short discussion of the nature of muscular skill. What does a skilful muscular movement feel like to the performer himself: how does he control it as he proceeds: how does he learn it: how does he remember it: how does he reproduce it? We know that when any particular task is undertaken, any particular movement made, a stream of messages is sent out in appropriate sequence, most accurately adjusted, along tens of thousands of nerve fibers that carry it out. We may imagine as a first and rough

approximation that the brain and nervous system contain a carefully catalogued set of gramophone records, each ready to be taken out and turned on when required. If we come to hurdle the high jump record is required: when we come to a ditch, the long jump record. In a sense this simple idea is true. Our behavior is largely composed of ready prepared, gramophone records called up and set going by the appropriate stimuli, and our skill in movement depends very largely on the degree to which we have learned to make it automatic. Nearly everything we do is partly unconscious. When we walk down the road we just give our nervous system general instructions to walk. We don't set out in detail how every muscle shall move, how every unevenness is to be overcome, we probably don't even know. Our movements, therefore, are largely automatic, they consist largely of gramophone records, prepared before hand by instinct or by training and ready instantly to be turned on. On the number of the actions which we have learned to make automatic, on their coordination one with another, on the fineness and accuracy with which they are adjusted to the stimuli which evoke them, depend largely the skill and efficiency with which we work. True as this is, however, it represents only a very limited aspect of the truth, because it neglects one half of the nervous system, the half that tells us what we are doing and enables us to adjust it as we go, the half by which we really remember what muscular movement is like.

Take the simplest possible movement. Try to bend your finger slowly at a uniform constant speed. You will find that it does not really move uniformly but in a rapid series of jerks. A record may be made in some way, for example, by connecting your finger to a lever writing on a smoked drum, or by photographing a beam of light reflected from a little mirror stuck upon the knuckle. The record allows the jerks to be counted. If the jerks be few they will be large and the finger will seem shaky; if they be many they will be small and the finger will seem steady; the most skilful person, and the one with the steadiest hand, is he who checks and controls his movements most frequently and rapidly. In all our movements the mechanism "hunts" to find the right adjustment. The smaller the amplitude of the "hunting" the more skilful we are.

How is this done? From all the moving parts of the body, muscles, tendons and joints, a system of nerves runs inwards carrying information about what is happening in those moving parts. These messages are started off by end organs, excited by movements in the tissues where they lie. Muscles are arranged in pairs or groups, and any given movement is due to the cooperation or antagonism of a whole set of different muscles. When you sail a boat you don't just set the sails and tie up the rudder: you watch the wind, you adjust the sheet, you keep your hand upon the tiller, there is a continual interplay between the wind, the sea, the sails and the steering. In the case of bodily movement the nervous system is the steersman, who has to compound all the messages—the nerve waves—he receives to form one general impression on which to act. When a given muscle shortens, its antagonist has to lengthen. But notice: the antagonist does not let go all at once: if it did, the result would be like letting the sail out with a crash: it pays out gradually, in little jerks, each element in the contraction of one muscle provoking reflexly an element in the relaxation of its antagonist. This interplay is going on continually, one muscle hauling in a little, another paying out, so guiding and controlling the movement, keeping it as smooth, as accurate and as well coordinated as may be. This continual reaction between muscles, nerves, end-organs and central nervous system is the physiological basis of muscular skill, and on its smooth and efficient working depend many of the things that mankind finds worthy of accomplishment.

I will not venture inside the nervous system itself. That would require not a lecture but a series of lectures to discuss. You must think of the nervous system as a vast exchange, in which incoming messages from all corners of the body are sorted, assessed, correlated, recorded, in which the response most necessary in view of all the circumstances is worked out. The automatic telephone exchange, the control post of the automatic traffic signal, both carry out this function in respect of a single purpose: the central nervous system does it in respect of all possible purposes. I can do little more in this lecture than refer to the messages themselves, those which come in, those which go out, those which—in all probability—circulate within the exchange itself. These messages are all of one kind, and before we can begin to understand the nature of nervous action it is necessary that we should know something about the unit on which the whole of the mechanism is based, the wave which travels in the thread of living protoplasm.

The ending of every sensory nerve fiber has a specific kind of sensitivity, to touch, to pressure, to heat, to cold, to light, to vibration, as the case may be, and its manner of reacting also is specific. Certain properties, however, are common to all. Of these the most significant is the cycle of refractoriness and returning excitability which follow. All these organs are "relaxation oscillators" of the type I referred to earlier. The specific stimulus is the external cause or constraint which alters the level at which spontaneous discharge takes place: the stronger the stimulus, the

lower the level, the more frequent the discharge. Some of these organs "adapt" to a constraint or stimulus. The hand put in warm water feels it warm, but in a very short time the organs sensitive to warmth adapt and no sensation is experienced. Similarly with touch. With sight there is some adaptation but not to the extent of complete lack of sensation: with the organs which tell us the state of tension in our tendons there is little: with sound there is practically none. The mechanism of this adaptation, this change of level of touch, is not understood, but its existence is an essential part of our being. The level of discharge of some of our oscillators is not constant under a constraint but gradually rises; with these the frequency of discharge diminishes, with others the level remains constant for long periods and so the same frequency persists.

One limit to the nerve fiber and the wave that travels in it as a means of reporting to the nervous system events occurring outside is that it can not react (in the case of man) more than about 1,000 to 2,000 times per second and at that speed only for a short time. Two of the most important stimuli are those of light and sound: *light* waves have a frequency of the nerve fiber; to limit appreciation of *sound* below 1,000 to 2,000 waves per second would greatly interfere with its equality. Now if a single nerve fiber, with no specific properties, is stimulated at a frequency higher than it can follow, two things may result: (1) It may respond at the highest submultiple of the frequency which it can follow, *e.g.*, $^1/_2$, $^1/_3$, $^1/_4$: (2) it may fail to respond at all. It will do the first if the frequency is not too high: the second if it is. It is well known that very high frequency stimuli (say 100,000/sec.) have no excitatory effect at all, even when of a strength many times greater than that of an ordinary alternating current. Sparks may be drawn from a man's hand with no more sensation than that of the heat produced by the current. This is commonly attributed to the fact that high frequency currents tend to travel on the surface of a conductor: the explanation will not hold, for two very good reasons: (1) the sensitive end organs are largely in the surface, so the effect should be greater, not less, and (2) with the high specific resistance of living material the skin effect is negligible, even at frequencies hundreds of times greater than those at which the excitatory effect disappears. The absence of stimulus is probably due to an electrical capacity existing at the surface of the excitable tissue, which prevents a potential difference from developing and a current from running across the surface until the capacity is charged. Whatever the explanation, the fact is a very obvious one: a nerve does not respond at all to stimuli of high frequency, which is probably why the limit of our hearing is about 30,000 waves per second. Below this limit, although an auditory nerve fiber is incapable of following say 10,000 vibrations per second, it can follow some submultiple of it, say 1,000 per second, and since the different fibers will very soon be out of phase with one another one tenth of them will respond to every cycle and the brain will in fact receive 10,000 volleys of waves per second. A number of nerve fibers can in this way respond to a frequency to which the individual fibers can not conceivably respond—a fact which has recently been verified by placing electrodes upon the auditory nerve and leading off to a loud speaker, when the ear and nerve together act as an efficient microphone, recording frequencies which no single fiber could conceivably follow.

In the case of light nothing of the kind occurs nor presumably could it with electromagnetic waves except of very great wave-length, and no specific receptor for these exists. In the case of light a chemical reaction occurs between the physical factor—the electromagnetic vibration—and the response evoked by it. Unlike the case of sound, there is no direct numerical relation between the wave-length of the light and the response evoked. The stimulus is turned into chemical code in the retina, and forwarded by waves of various frequencies and in various fibers to the brain .There it is interpreted. The ear is capable of responding to frequencies from 50 to 20,000 or so, a ratio of 400 to 1, a matter of nearly 9 octaves. The eye responds only within one octave. The difference presumably is that the nervous mechanism is capable of transmitting the response to the separate sound waves as separate groups of conducted nerve waves to the brain; the light waves have to go by a roundabout way *via* a chemical reaction and a code. What a wonderful world of color we should have were our eyes sensitive to nine octaves!

One of the greatest achievements of physiology in the last years has been the proof by Adrian and his colleagues that the only way in which a sensory nerve fiber alters its response, as the strength of the stimulus is altered, is by a change in the frequency of the waves which it forwards to the central nervous system. I may have disguised my meaning by a too careful choice of words, but I fear the wrath of my psychological friends if I dare to hint that the sensation due to a stimulus is measured by the frequency of the nerve waves which it produces. The total effect of a stimulus depends upon three factors: the number of fibers which, by means of their end organs, it affects: the frequency of the waves evoked in each of these fibers: and the state of the nervous system, as affected by other stimuli simultaneously occurring, and other such factors.

If I am to keep outside the central nervous system I must never come to sensation at all, but refer only to the number of separate nerve units reacting to a stimulus and to frequency of the reaction of each. The waves in these fibers are, however, at least the physiological basis of sensation.

At the other end of the scale is the response of muscles and glands by which the purposive reactions of the nervous system are affected. Here again waves in nerve fibers are the means by which communication is effected and orders despatched. These are waves of the same identical nature as occur on the sensory side, but waves of the type occurring in nervous conduction can never cross one another, one-way traffic is necessary, and separate motor nerve fibers are provided. Here let me make a digression for a moment. A wave leaves a discharged region, a refractory region, behind it; for the same reason as waves can not follow one another with less than a certain interval two waves can not cross—each comes into the discharged region of the other and both are wiped out. Curiously enough, however, all tissues capable of conducting these waves are in fact able to do so equally in either direction: though in normal life, except in special instances (and perhaps in the central nervous system itself) they never do. Sometimes tissues are so arranged that a wave can take on a circus movement and go on running round—chasing its own tail—more or less indefinitely: this curious phenomenon, discovered in the heart of a marine animal, is the basis of an important clinical disorder of the human heart. In the nerve networks of some primitive animals, and in those of the vegetative organs of higher ones, the same type of circus movement of nerve waves probably occurs. Mainly, however, one finds waves which begin and end in a single stretch of conducting tissue.

These waves, which are orders to the effector organs, reach in general three types of reacting cells—voluntary muscle cells, involuntary muscle cells, and gland cells. Their passage to the voluntary muscle cells is so rapid that one tends to regard it as of the same nature as conduction in the nerve itself. In the other two it may have as intermediary, at least in certain instances, the liberation of a chemical substance—*e.g.,* acetyl choline or adrenaline—which slowly produces a more prolonged response. The manner and mechanism of conduction from nerve to muscle fiber is still disputed, but there are some interesting facts to record.

Let me first, however, refer to muscle and its response. The elementary unit of muscular contraction is the twitch—a rapid wave of shortening—lasting only a few hundredths of a second in a human voluntary muscle. All ordinary contractions are made up by the fusion of such twitches: and the strength of a contraction depends upon the number of fiber groups reacting and the frequency with which these react. A maximum effect is made with 50 motor impulses per second. This frequency can, as a matter of fact, be rather easily seen in a muscle by recording either its mechanical or its electrical response in a strong contraction. In a weak contraction the fibers are reacting at a lower frequency and out of phase with one another, so not much is seen. In a strong contraction groups of them seem to fall into phase by some kind of resonance in the central nervous system. Normally the impulses pass from nerve to muscle without hindrance. In some conditions, however, a block is interposed across which the waves can pass only with difficulty, if at all.

Such a condition may exist in a normal muscle after severe fatigue: experimentally it is best seen by poisoning with the South American arrow poison, curare. In this state the first effect is that only low frequency impulses can be transferred from nerve to muscle: the next is that complete paralysis occurs. The mechanism of this paralysis is disputed, but one curious and interesting example of it occurs in man. A disease exists called *myasthenia gravis,* in which the patient is incapable, for more than a second or two, of making any considerable effort. If the ulnar nerve in the human arm be stimulated with a series of electric shocks the muscles working the fingers are thrown into contraction. In a patient with myasthenia two things may happen. If the shocks are of rather high frequency the response rapidly fades away. If they are of low frequency the response is nearly normal. When failure to respond to a higher frequency has occurred, transfer to a lower frequency gives the full response again. Here apparently there is an abnormal condition in nerve or nerve-ending which is probably the cause of the characteristic sign of the disease. Few physiologists are so sour that such a sudden contact with clinical medicine does not give them a thrill of pleasure. Such contacts, seemingly by chance, provide even the most academic science its relation to reality.

Apart from the ordinary motor and sensory impulses to which I have referred, a multiplicity of mechanisms exists all over the body, depending on the use of impulses in nerve fibers for coordinating function. The lungs, for example, expand and contract rhythmically. This depends partly upon a genuine rhythmic function in the center—a spontaneous discharge of waves from certain cells. On this, however, a major control is exerted by impulses sent from end organs in the lungs themselves. The movement of the lungs excites these organs which discharge waves along fibers to the center and so check and control and

limit the contraction of the diaphragm. Normal breathing is a balanced movement consisting of the continual interplay and adjustment of impulses going to and from the center. Another such mechanism exists in the carotid sinus by which the pressure and composition of the blood cause impulses to be discharged to the centers by which adjustments of these are effected. With the high power of modern electrical instruments there is scarcely any corner of the body from which such messages can not be found pouring out—and listened to with loud speakers, recorded with oscillographs.

You probably have heard the story of the little boy who wanted to know how animals came into existence and had been told by his mother that God had made them. "And did God make flies, too, Mummy?" "Yes, dear." "A fiddling job making flies." If he could have connected loud speakers and oscillographs on to the nerve cells and nerve fibers of a fly and heard and seen the amazing complex of wave motion, roaring backwards and forwards even in that small creature, he would have realized that "fiddling" was far too mild an adjective. I do not know how many motor and sensory medullated nerve fibers there are in a man, but let us guess. Assume that they weigh altogether 100 grams, that they have an average length of 50 cm and an average diameter of 14 μ. This would give about a million of them: I expect this is an underestimate. Let us think of a man running in a quarter mile race, exerting himself to his utmost. I think we may admit an average of at least 10 impulses per second to each of the fibers; total, 10 million waves per second. This hurricane of coordinated impulses is raging in our runner. Even at rest I suppose we may allow him one twentieth of this, half a million per second. Each of these impulses could be picked up and recorded or made audible by modern electrical technique. Each wave has the same general characteristics. Each is a reasonable and intelligible thing. The properties of a gas depend upon the average behavior of its molecules, but that is governed by statistical rules. The properties of a man depend upon the total behavior of his nerve impulses—but these—although so many—are coordinated and adjusted. Truly, as David said, we are fearfully and wonderfully made.

(Address before the Century of Progress Meeting of the American Association for the Advancement of Science, June, 1933, which is being published in the forthcoming number of The Scientific Monthly. It is substantially the same as the lecture which Dr. Hill gave at the Marine Biological Laboratory during his brief visit to Woods Hole early in July.)

A CATAPHORESIS CHAMBER FOR HEAVY OBJECTS

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AND

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With numerous heavy objects such as Arbacia eggs the force of gravity places serious difficulties in the way of taking cataphoretic measurements in a horizontal cataphoresis chamber such as that described by Northrop (1922. *J. Gen. Physiol.* **4**: 629). The writers have done some preliminary work on developing a method for obtaining these measurements. An attempt has been made to take direct measurements which do not involve such mathematical calculations as the use of the horizontal component of motion (K. Dan, 1931. *Anat. Rec.*, Suppl., **51**: 28).

Accordingly, a vertical chamber, very much smaller than the ordinary one, was constructed. In this vertical chamber the impressed electromotive force is parallel to the force of gravity. By impressing a suitable E. M. F. it is possible, when this is opposed to gravity, to obtain a null point at which the two forces are exactly balanced; the object under consideration is then held motionless by the opposing forces. Its velocity under the influence of gravity alone and under that of gravity plus the E. M. F. necessary to establish a null point can then be measured. From theoretical considerations, the latter should be twice the former; from the material examined, this seems to be the case. The electrical charge on the surface of the object can then be calculated by the Helmholtz-Lamb equation.

The cataphoresis chamber itself was made by compressing a section of a piece of pyrex glass tubing. Platinum calibrating electrodes were inserted at the top and bottom ends of the chamber. The chamber was placed in a water bath to overcome heating effects. It was found that the chamber used (approximately 1.6 cm. x 0.9 cm. x 0.25 mm.) was more satisfactory than larger ones due to the fact that its small size to a great extent eliminated convection currents. The chamber, inside its water-bath, was observed through a microscope with a 16 mm. objective.

From results obtained by cataphoretic studies on the egg of *Arbacia punctulata* it seems apparent that the method outlined above is a feasible one for such work. It is planned to continue this work with modifications of the chamber, among them a chamber made of capillary tubing.

In conclusion, the first named writer wishes to express his gratitude for a Collecting Net Scholarship which enabled him to spend the summer of 1933 at the M. B. L. taking the course in Physiology and participating in beginning the above outlined research.

THE BIOLOGICAL LABORATORY
COLD SPRING HARBOR

ANOMOLOUS OSMOTIC PRESSURES OF COLLOID SOLUTIONS AT EQUILIBRIUM

David R. Briggs

Negative osmosis, a special case of anomalous osmosis, was described by Detrochet[1] long before the true nature of osmotic pressure itself was understood. His observations were made using pig's bladder as the membrane with dilute acid solutions and water as the liquids. He found that the *flow* of liquid was away from the acid solution into pure water. Graham[2] made similar observations as also did Flusin[3]. The latter sought an explanation of the phenomena by analogy with the preferential passage of liquids through a rubber membrane which was bathed on the two sides by different liquids. He advanced the idea that the inbibition pressure (swelling pressure) of the membrane, by differing in the two liquids or solutions bathing it, could account for anomalous osmotic flow through the membrane.

Girard[4], Loeb[5] and Bartell[6] have shown the possibility of an electrosmotic force superimposing itself on the true osmotic forces to cause anomalous osmotic flow. Loeb used collodion or collodion coated with protein as membranes, and with dilute salt solutions showed a definite correlation between the electrokinetic properties of the membrane and the rate of osmotic flow of liquid through the membrane.

Most cases definitely recognized as anomalous osmosis have been observed in systems similar to that mentioned above, wherein the osmotically active materials are electrolytes to which the membrane is slowly permeable. In such systems it is not possible to obtain equilibrium data because equilibrium is attained only when the concentration of the electrolyte is equal on both sides of the membrane. However, if the system contains one ion to which the membrane is not permeable, while it is permeable to all other ions and molecules present, it becomes possible to obtain equilibrium data. Numerous observations have indicated that even at equilibrium anomalies exist in the osmotic pressures shown by colloid solutions, all of which can not be explained in terms of Donnan's theory of membrane equilibrium in such systems. Any colloid electrolyte present on only one side of a membrane fulfills the requirements mentioned.

Duclaux[7] studied the osmotic pressure of solutions of iron and thorium hydroxide, prussian blue, copper ferrocyanide, gum arabic and caramel, using collodion membranes. Donnan's theory of membrane equilibrium was not yet known and its influences on osmotic pressures were not recognized by Duclaux. He found that the osmotic pressure at equilibrium was always lower than that to be expected on the basis of the specific conductivity of the colloid solutions, and that, as the concentration of the colloid was increased, the osmotic pressure increased faster than a direct proportion relationship required.

Loeb[8] studied the osmotic pressure in such systems when he measured that of solutions of various proteins. He found that the difference in concentration of crystalloid ions on the two sides of the membrane at equilibrium set up as a result of the Donnan phenomena could account completely for the osmotic pressure shown by a solution of casein chloride[9], for example. In this case, the colloid micelle was apparently so large that it exerted no measurable osmotic pressure itself. With gelatin chloride, Loeb found that the osmotic pressure observed did not coincide with that calculated from the difference in ionic concentrations, although it varied in much the same manner with change in pH of the system. Loeb believed that the osmotic properties of protein solutions were completely defined by the Donnan hypothesis.

E. Hammarsten[10] found that the osmotic pressure exhibited by nucleic acid and Na_4-nucleate inside a collodion membrane, against distilled water on the outside was less than that which the equilibrium distribution of small ions alone should show, also less than would be expected if no ionization occurred and the material were molecularly dispersed (calculated from the known molecular weight of the acid). He concluded, therefore, that some aggregation had taken place and that the small ions are active osmotically only to a small extent or not at all. This loss of osmotic activity of the small ions (which, however, still retain their activity at an electrode, since their concentrations were determined in this manner) he assumes to be due to an interionic influence resulting from the great difference in size of the colloid and crystalloid ions. In support of this explanation H. Hammarsten[11] found that the osmotic pressure of a

solution of guanylic acid (ion of relatively small molecular weight) was that normally expected from the difference in total ionic concentration on the two sides of the membrane. (His calculations were made from the value of a maximum osmotic pressure observed after about six hours). With salts of acids of higher molecular weight he confirmed the observations of E. Hammarsten. This anomalous osmotic effect, wherein the osmotic pressure of the colloid solution is less than would be calculated on the basis of the measured activities of the components present, has been called the "Hammarsten effect."

Similarly Donnan and Harris[12] and Bayliss[13] had found for solutions of congo red that the observed osmotic pressure is only 87-94% of that calculated on the assumption of a molecularly dispersed but unionized state of the dye. Donnan assumed the formation of aggregates of dye molecules. Zsigmondy[14] measured in addition the conductivity of the colloid solution and concluded that the relationship between conductivity and osmotic properties, even with the assumption of the formation of a large colloidal ion, could not be explained on the basis of the older theories. He postulated that the discrepancy arises as a result of interionic forces in the manner pictured by the Debye-Hückel theory.

Bjerrum[15] measured the osmotic pressure, membrane potentials, and H^+ ion activity of solutions of chromium hydroxide and found that $P = P_1 + P_2$, where P was the observed pressure, P_1 was the osmotic pressure of the colloid particles (estimated to contain about 1000 Cr atoms) and P_2 was the osmotic pressure derived from the unequal distribution of ions on the two sides of the membrane. He found discrepancies between the observed membrane potentials and those calculated from osmotic pressure measurements, however, and believed this to be due to an unequal influence of the colloid upon the conductivity, activity, and osmotic action of ions adsorbed by it.

Samac, Knop, and Pankovic[16] measured osmotic pressures of an amylopectin (from potato starch), of ligno sulfuric acid, and of an ammonium salt of humus (from peat) against water, using collodion sacs. They found the observed osmotic pressures to be less in all cases than would be expected from the measured difference in concentration of the small ions alone and to be of the order of magnitude to be expected from the molecular weights of the colloids, calculated from other data, entirely neglecting any ionization. Their conclusion was that the small ions (*Gegenionen*) were *osmotically* inactive. Samac and Ribaric, however, had found that the freezing point lowering in a ligno sulfuric acid solution was very close to that required on the basis of hydrogen ion concentration alone.

Adair[17] studied the osmotic pressure of haemoglobin against distilled water and against solutions of diffusible salts. He measured, at the same time, the membrane potentials and distribution of diffusible ions. His conclusions were (1) that the relationships between diffusible ion activities and membrane potentials are in accord with the Donnan theory, (2) that within a limited range of hydrion, salt, and protein concentrations the observed osmotic pressure was equal to the sum of the pressure derived from the colloid, molecularly dispersed, and the pressure arising as a result of unequal distribution of diffusible ions on two sides of a membrane, and (3) that the theory of Barcroft and Hill[18], which pictures a variable degree of aggregation of the colloid in explanation of the anomalies observed in its osmotic pressure relationships, was not correct. Adair claims the large deviations from the van't Hoff law to result from changes in activity of the protein molecule, this being a function of salt concentration, hydrion concentration, and protein concentration.

That osmotic pressure measurements on solutions containing colloid electrolytes are difficult of interpretation seems obvious. That some unrecognized force may be acting seems probable. The following extensive data obtained on such a system may serve to throw some light upon the course, if not the cause, of these anomalies in colloid osmotic pressures observed at equilibrium.

Experimental

The colloid used in these experiments was gum arabic prepared from selected sorts. These were dissolved to make about one per cent solution in water, the solution was filtered, and sufficient concentrated HCl was added to make the solution about N/10. The gum was then precipitated with alcohol. It was dissolved and precipitated a second time and the gum was dried in vacuo to drive off most of the alcohol, then redissolved in water and electrodialysed against many changes of water (in outer compartments of the three compartment electrodialysing cell) until no further change in conductivity of the solution occurred. The electrodialysis required about a week's time, the current being maintained at about 0.3 amps. until the resistance of the water in the outer compartments would no longer carry this amperage at 110 volts D. C. An appreciable amount of $Ca(OH)_2$ precipitated out in the cathode chamber during this process showing that the alcohol purification of the gum had not removed all of the cations present. No hydrolysis of the gum occurred.

After electrodialysis, a sample of the gum was dried and ignited. Only a trace of ash, (ca. 0.05%) was detectable. When a sample of the purified gum was titrated with standard NaOH or Ca $(OH)_2$ to a pH of 7, and then dried and weighed, it was found that each gram of arabic acid required 85 x 10^{-5} equivalents of alkali to neutralize it. The titration curve was that of a fairly strong acid (pK = 3.3 when concentration was 100 gm. per liter of water) and agreed fairly closely with that reported by Thomas and Murray[19]. The equivalent weight of the gum arabic studied by them was the same as that used in the

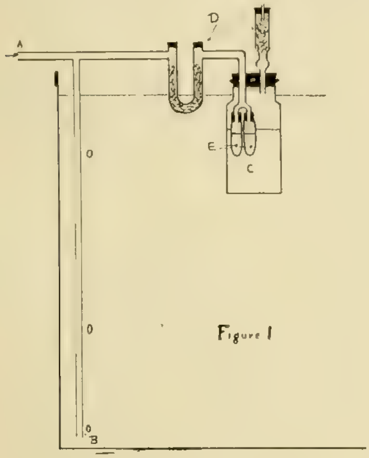

Figure 1

present experiments. The stock solution of arabic acid was neutralized with the hydroxide of the metal, the salt of which was desired and completely dried in vacuo at 67° C, and kept in a desiccator until needed.

The method used for measuring the osmotic pressures of the solutions was that of applying a constant pressure to the colloid solution inside of a sac shaped membrane which was partially or wholly immersed in an external solution containing no colloid, until equilibrium was attained throughout the system. Figure 1 shows diagramatically the apparatus used. The samples of colloid to be examined (in duplicate) was placed in collodion sacs (E) which were fastened by rubber bands to the tube through which constant pressure was applied. The applied constant pressure was obtained by slowly forcing air into the system at (A) and allowing it to escape at (B) thereby displacing water in the tube between (B) and the surface of the liquid external to the tube. The pressure effective on the miniscus of the colloid solution in (E) would then be equal to the column of water displaced in the tube (A to B) modified by the difference in the height of the minisci in (E) and in (C). Most of the experiments were carried out when the pH was well on the acid side of neutrality in order to eliminate CO_2 adsorption. In those cases where neutrality was approached in the solutions, CO_2 adsorption was reduced to a minimum by protecting both sides of the system containing the colloid and external solutions by soda lime tubes (D). This protection was imperfect, however, and in a few cases where the external solutions is near neutrality, it is necessary to assume that some HCO_3^- ion is present.

The final volume containing the colloid was determined by weighing the solution left inside the membrane at equilibrium. The observed (equilibrium) osmotic pressure, then, would be equal to the constant pressure applied. The final concentration of the colloid in solution could be calculated from the amount of colloid initially placed in the sac and its volume as determined from its final weight. The final volumes for the colloid solutions used in the following tables of calculations are corrected for the weight of gum arabic present in each instance (i. e., V_i = F — A). The membranes used were made of collodion. They were prepared in the usual manner, no particular care being exercised as to uniformity of their preparation. The only care exercised in their preparation was that required to get them thick enough to withstand the pressure applied and to prevent leaks, but not so thick or impermeable as to require too long a time for equilibrium to be reached. Table I illustrates the fact that equilibrium values obtained are independent of the pore radius and material of the membrane.

In all experiments the determinations were made in duplicate by placing equal amounts of colloid in two sacs which were then placed under the same pressure with a common external solution. The final weight of solution in each sac is given in the tables in the column designated by F, the weight of dry colloid contained in each is given in the column under A. Subsequent measurements of pH and all calculations are made upon the combined samples. In all experiments, except those given in Table II, the external solutions were varied as to salt content, pH, or some other factor. These measurements were carried out in individual 500 cc. bottles, as shown in Fig. 1.

The weights of water present in the internal (i) and external (o) solutions at equilibrium are given in the tables in the columns designated as V, and the pressure against which equilibrium was attained by P_o.

After equilibrium had been gained, the H^+ ion concentration was determined electrometrically (with the hydrogen electrode) on the inside and outside solutions. These measurements gave

directly the difference in potential, E, across the membrane, and from them could be calculated the ratio of distribution of hydrogen ions in the system. This ratio is shown in the tables as R. The total equivalents of Na and Cl present in the system in each case is shown in the tables under columns headed by Na conc. and Cl conc. respectively. Since, according to Donnan's membrane equilibrium theory,

$$\frac{[H^+]_i}{[H^+]_o} = \frac{[Na^+]_i}{[Na^+]_o} = \frac{\sqrt{[Ca^{++}]_i}}{\sqrt{[Ca^{++}]_o}} = \frac{[Cl^-]_o}{[Cl^-]_i} = R$$

this value of R together with a knowledge of the volumes, V, inside and outside, and the total equivalents of each species of anion or cation present in the system, makes it possible to calculate the actual concentrations of each on both sides of the membrane. These values are calculated assuming 100% activity in all cases except where definitely indicated otherwise. The equilibrium concentrations of the various diffusible ions on the two sides of the membrane were calculated according to equations such as

$$[Na^+]_o = \frac{\text{Total equivalents of } Na^+}{(R \times V_i/_{1000}) + V_o/_{1000}} \quad \text{and} \quad [Na^+]_i = R \times [Na^+]_o$$

$$[Cl^-]_i = \frac{\text{Total equivalents of } Cl^-}{(R \times V_o/_{1000}) + V_i/_{1000}} \quad \text{and} \quad [Cl^-]_o = R \times [Cl^-]_i$$

where V_i and V_o = equilibrium volumes of solvent inside and outside, respectively.

The electrolyte concentration in all cases where 100% ionization is assumed is of such a low order that the error so introduced is very small. In the tables, the concentrations of these ions are given under the heading $[Na^+]$, $[Cl^-]$, $[H^+]$, etc. and signify the concentration in equivalents per liter multiplied by 10^7. If the Donnan equilibrium theory holds in these cases, the total concentration of the positive ions should equal that of the total negative ions in the outside solution where diffusible electrolytes alone are present. In the majority of cases this agreement is close, and the conclusion is that the ions distribute themselves in accordance with the membrane equilibrium theory.

Knowing, now, the actual concentration of all ions on both sides of the membrane (neglecting, for the present, the colloid ion, since its molecular weight is high and unknown), the difference, (D), between their totals, (T), inside and outside, will be a measure of the difference in their osmotic concentrations across the membrane. The equilibrium measurements were all made at 25°C and at this temperature one mole of dissolved material exerts an osmotic pressure equal to 25280 centimeters of water. Then (D) x 25280 x 10^{-7} gives the calculated osmotic pressure, P_c, in cm. of water, exerted by the ions, assuming that the large colloid ion itself exerts no appreciable osmotic pressure and that it does not influence the osmotic activity of the small ions to a degree different from its influence on the activity of these ions as determined electrometrically (i. e., from H^+ ion activity measured electrometrically and values calculated therefrom in the above outlined manner).

Examination of the tables reveals that in all cases (except for higher concentrations of $CaCl_2$ in the Ca^{++} series) the values of P_c are greater than the corresponding values of P_o. This shows that the osmotic pressure arising from the unequal distribution of the small ions alone is greater than the observed osmotic pressure. This is in accord with observations referred to above in the cases of various other colloid solutions. Any osmotic pressure arising from the colloid ion itself would be in a direction such as to increase this difference. The value, $P_c - P_o = P_x$, is a measure of the anomalous equilibrium pressure of the colloid solution (continuing to neglect any possible osmotic pressure derived from the colloid itself).

The Na salt of arabic acid was the colloid used in all experiments except those of Table VI, where the Ca salt was used.

In the experiment shown in Table II, the vessel used to contain the external solution was a tall glass cylinder. The external solution was common to all the sacs. Pressure was varied by placing the sacs at different levels below the surface of the liquid and applying an equal external pressure to all. The effective pressure then would be the difference between the applied pressure and the counter pressure exerted by the depths of water above the miniscus of the colloid solution in the sac, i. e., the weight of a column of water equal to the distance of this miniscus below the surface of the liquid in the containing cylinder.

For Table III the variable was the pH. Experiments were made individually. The total Cl content was kept constant and the pH varied by varying the relative amounts of NaCl and HCl added. In this case, and in all those in succeeding tables the value of P_o was held nearly constant.

In experiments of Table IV, all variables were held constant except ethyl alcohol content, which

TABLE I. VARIOUS MEMBRANES—TEST FOR CONSTANCY OF (r).

#	Type of Membrane External soln.	A gm.	F gm.	P cm.H_2O	V (i + o) cc.	EMF mv.	Remarks
a 1b out	3 minute dry collodion thin cellophane distilled water	.150 .150	7.672 7.633	130.0 130.0	252	No measurements made	Formed from sheet cellophane
a 2b out	3 minute dry collodion 12% guncotton in CH_3COOH distilled water	.200 .200	10.487 10.292	129.1 129.1	252		Formed in testtube and washed for 2 days in distilled H_2O before use
a 3b out	3 minute dry collodion S&S parchment thimble distilled water	.150 .150	7.625 7.690	129.2 129.2	252		Thoroughly soaked and washed before use
a 4b out	3 minute dry collodion 10 minute dry collodion distilled water	.175 .175	9.380 9.305	130.4 130.4	320	544.1 545.3 685.5	Membrane was dried 10 min. (in tube) before placing it in H_2O
a 5b out	3 minute dry collodion 20 minute dry collodion distilled water	.175 .175	9.332 9.197	130.5 130.5	320	543.9 542.7 672.0	Membrane was dried in tube (in which it was formed) for 20 min. before placing in H_2O
a 6b out	3 minute dry collodion 60 minute dry collodion distilled water	.175 .175	9.387 9.920	130.9 130.9	320	540.3 679.2 659.5	Membrane was almost impermeable to water. It was dried in stream of air for 1 hr. before being placed in H_2O

Legends For The Tables

A = grams of arabate in sample.

F = final weight of solution containing sample inside of membrane.

V = volume of water at equilibrium on each side of membrane (cc.)

P_o = observed (or equilibrium) osmotic pressure (cm. H_2O).

$[H^+]$, $[Na^+]$, etc. = final concentration of diffusible ions (equivalents per liter x 10^7).

E = membrane potential = 59.1 x(pH_o—pH_i) millivolts).

R = ratio of distribution of diffusible ions across membrane.

[T] = total concentration of diffusible ions (equivalents per liter x 10^7) on each side of membrane.

[D] = difference in conc. of diffusible ions (equivalents per liter x 10^7) across membrane.

P_c = calculated osmotic pressure = [D] x 25280 x 10^{-7} (cm. H_2O).

$P_x = P_c - P_o$.

a = percentage ionization of arabate.

$[—]_i = [Cl^-]_i + [HCO^-_3]_i$ (equivalents per liter).

was varied from 0.25 x 10^{-3} molar to 0.25 molar. This experiment was made in order to find out if nonelectrolyte had any effect upon the osmotic properties of the colloid solution. These results serve, perhaps, to show the extremes of error in the determinations and in the calculated value of P_x. This is of the order of 10%, the alcohol apparently having no effect upon the equilibrium attained.

(Continued on Page 281)

TABLE II. PRESSURE VARYING. SAME EXTERNAL SOLUTION

	Side of Membrane	In total soln. — 5000 cc. (i + o) Cl conc. eq x 10^3	Na conc. eq x 10^3	A gm.	F gm.	V cc.	P_o cm. H_2O	H+ ion conc. EMF mv.	pH	$[H+]$ x 10^7	E mv.	R	$[Na+]$ x 10^7	$[Cl-]$ x 10^7	$[T]$ x 10^7	$[D]$ x 10^7	P_i cm. H_2O	$P_i - P_o$ cm. H_2O	α	$\frac{E\alpha}{Px}$
1	i	4.513	2.550	.100 .100	5.201 5.029	10.030	14.5	405.8	2.722	18960	23.9	2.530	20050	6050	45060	14331	36.3	21.8	0.1945	0.2132
2	i	4.513	2.550	.100 .100	3.829 3.848	7.447	27.3	401.0	2.639	22950	28.7	3.061	24260	5000	52210	21481	54.2	26.9	0.1855	0.1980
3	i	4.513	2.550	.150 .150	4.865 4.840	9.405	37.8	397.3	2.578	26420	32.4	3.525	27930	4343	58693	27964	70.7	32.9	0.1844	0.1815
4	i	4.513	2.550	.150 .150	3.965 4.245	7.910	48.7	395.0	2.540	28830	34.7	3.847	30490	3980	63300	32571	82.4	33.7	0.1717	0.1767
5	i	4.513	2.550	.200 .200	3.850 3.836	7.296	85.7	389.4	2.443	36050	40.3	4.810	38110	3184	77344	46615	117.8	32.1	0.1522	0.1909
6	i	4.513	2.550	.200 .200	3.534 3.518	6.652	97.4	387.1	2.404	39430	42.6	5.260	41700	2914	84044	53315	134.8	37.4	0.1531	0.1744
7	i	4.513	2.550	.300 .300	4.961 4.915	9.276	110.5	385.5	2.377	41960	44.2	5.595	44350	2735	89045	58316	147.5	37.0	0.1519	0.1815
8	i	4.513	2.550	.300 .300	4.673 4.784	8.857	119.5	384.4	2.358	43830	45.3	5.850	46360	2617	92807	62078	157.1	57.6	0.1520	0.1832
	out					2933.1		429.7	3.125	7495			7924	15310	30729					

TABLE III. VARYING pH. TOTAL [Cl] CONSTANT. PRESSURE CONSTANT.

#	Side of Membrane	In 320 cc.= Total vol. of soln. (i + o): Cl conc. eq. x 10^3	Na conc. eq. x 10^3	A gm.	F gm.	V cc.	P_c cm. H_2O	H+ ion conc.: EMF mv.	pH	$[H+]$ x 10^7	E mv.	R	$[Na-]$ x 10^7	$[Cl-]$ x 10^7	[T] x 10^7	[D] x 10^7	P_c cm. H_2O	$P_c - P_o = P_x$ cm. H_2O	α	$\frac{E\alpha}{P_x}$
1	i	0.680	0.340	.200 .200	2.589 2.583	4.77	127.1	379.4	2.273	53320	39.6	4.696	47320	4580	105220	62370	157.5	30.4	0.1348	0.1756
	o					314.83		419.0	2.945	11350			10080	21520	42850					
2	i	0.680	0.437	.200 .200	2.860 2.884	5.37	127.1	385.4	2.375	42170	40.1	4.786	61520	4505	108195	64955	164.2	37.1	0.1567	0.1694
	o					314.23		425.5	3.055	8810			12860	21520	43240					
3	i	0.680	0.534	.200 .200	3.161 3.235	6.00	127.6	393.8	2.519	30280	40.9	4.910	76400	4398	111078	67748	171.2	43.6	0.1805	0.1694
	o					313.60		434.7	3.210	6165			15565	21600	43330					
4	i	0.680	0.631	.200 .200	3.735 3.734	7.07	127.5	405.0	2.707	19630	42.0	5.166	93400	4191	117221	73691	186.2	58.7	0.2262	0.1620
	o					312.53		447.0	3.420	3800			18080	21650	43530					
5	i	0.680	0.728	.200 .200	4.567 4.742	8.91	128.1	422.5	3.003	9930	43.3	5.392	109470	4039	123439	79528	201.0	72.9	0.3025	0.1796
	o					310.69		465.8	3.735	1841			20300	21770	43911					
6	i	0.680	0.826	.200 .200	5.488 5.403	10.49	128.8	445.5	3.391	4062	46.5	6.150	136000	3558	143620	98970	250.0	121.2	0.4211	0.1618
	o					309.11		492.0	4.180	660.5			22110	21880	44650					
7	i	0.680	0.923	.200 .200	6.495 6.520	12.62	129.5	483.1	4.030	933.0	55.9	8.810	194500	2503	197936	153700	388.3	258.8	0.7170	0.1550
	o					306.98		539.0	4.975	105.9			22060	22070	44236					
8	i	0.680	1.020	.200 .200	7.288 7.272	14.16	129.0	587.4	5.793	16.10	59.2	10.08	229500	2200	231700	186770	472.0	343.0	0.9470	0.1637
	o					305.44		646.6	6.797	1.596			22750	22180	44930					

TABLE IV. ALCOHOL VARYING. [Cl] CONSTANT. PRESSURE CONSTANT. [Na] CONSTANT.

#	Side of Membrane	In 320 cc. Total vol. of soln. (i o) Cl conc. eq. x 10^3	Na conc. eq. x 10^3	Ethyl Alcohol Mols per liter x 10^3	A gm.	F gm.	V cc.	P_o cm. H_2O	H+ ion conc. EMF mv.	pH	[H+] x 10^7	E mv.	R	[Na+] x 10^7	[Cl−] x 10^7	[T] x 10^7	[D] x 10^7	Pc cm. H_2O	$P_c - P_o =$ cm. H_2O	α	$\frac{E\alpha}{P_x}$
1	i	0.3128	0.5930	0.25	.200 .200	8.330 8.382	16.36	130.2	511.3	4.505	312.5	75.0	18.52	181300	335.5	182200	162100	410.0	279.8	0.872	0.2338
	o						303.2		586.3	5.773	16.86			9790	10280	20100					
2	i	0.3128	0.5930	0.50	.200 .200	8.449 8.410	16.46	130.2	512.7	4.530	295.0	73.6	17.50	175500	587.0	176400	155800	394.0	263.8	0.848	0.2365
	o						303.1		586.3	5.773	16.86			10300	10280	20600					
3	i	0.3128	0.5930	5.00	.200 .200	8.353 8.288	16.24	129.8	510.8	4.500	316.2	72.5	16.80	172800	611.0	173700	153100	387.0	257.2	0.824	0.2322
	o						303.4		583.3	5.725	18.82			10295	10270	20600					
4	i	0.3128	0.5930	10.00	.200 .200	8.388 8.356	16.34	130.2	509.4	4.475	335.0	74.9	18.76	182400	548.0	183300	163300	413.0	282.8	0.875	0.2320
	o						303.3		584.8	5.748	17.86			9725	10290	20000					
5	i	0.3128	0.5930	25.00	.200 .200	8.442 8.370	16.41	130.2	511.1	4.502	314.7	75.2	18.66	181500	551.0	182400	162400	410.5	280.3	0.875	0.2348
	o						303.2		586.3	5.773	16.86			9730	10280	20000					
6	i	0.3128	0.5930	50.00	.200 .200	8.359 8.273	16.23	129.9	510.9	4.500	316.2	73.7	16.95	173750	607.0	174700	154200	390.0	260.1	0.829	0.2348
	o						303.4		583.6	5.730	18.65			10250	10280	20500					
7	i	0.3128	0.5930	250.00	.200 .200	8.519 8.504	16.68	127.2	513.9	4.549	282.3	74.6	18.23	178100	565.0	178900	158800	401.5	274.3	0.873	0.2374
	o						302.9		588.5	5.810	15.48			9770	10300	20100					

Table V shows results obtained when the total Cl was changed from zero to .01 normal solution. In this case a mixture of 5 parts NaCl and 1 part HCl was added to the individual experiments in varying amounts so as to change the total Cl. The HCl was added in order to keep the pH on the acid side of neutrality and minimize CO_2 absorption. In this table the activity coefficients of NaCl were used to calculate the concentration of Na^+ and Cl^- ions present, i. e., 100% dissociation was not assumed. This use of the activity coefficients of NaCl is not entirely correct (not all the electrolyte was NaCl) but serves to give a somewhat closer approximation to the actual conditions than if 100% ionization were assumed. Experiments in which the K and Li salts of arabic acid were used in place of the Na salt, and in which KCl and LiCl were used in place of NaCl, respectively, showed no lyotropic effect for monovalent cations, the values obtained being identical (within a few per cent) with those given in Table V for the Na salts. While not shown in the table, the Cl^- ion concentrations were determined electrometrically with silver—silver chloride electrode in the external solution and were found to agree closely with the calculated values given. The agreement for the inside solutions was not good, especially at low Cl concentrations, the electrode measurements indicating concentrations which were somewhat higher than the calculated values given in the table.

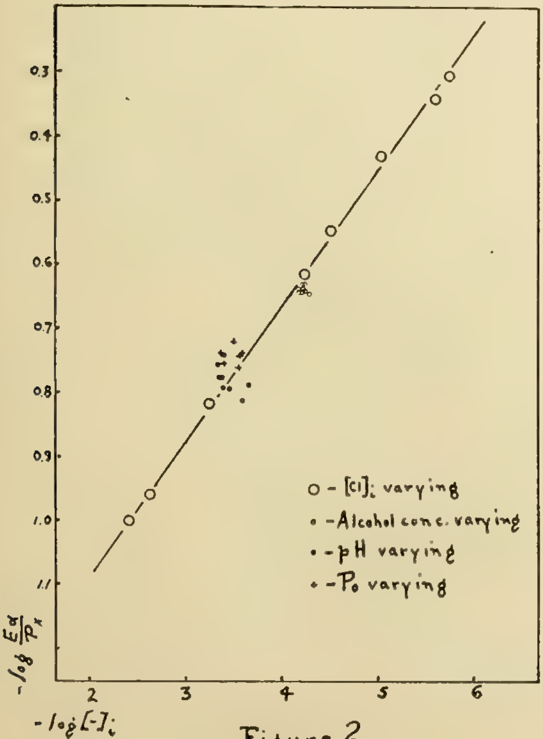

Figure 2.

In Table VI are shown the results obtained when the Ca salt of arabic acid and $CaCl_2$ were used in an experiment otherwise similar to that of Table V. In this case the Cl^- ion concentration outside the membrane at equilibrium was determined electrometrically and the equivalent Ca^{++} ion concentration outside taken to be equal to $[Cl^-]_o - [H^+]_o$. From the value of $[Ca^{++}]_o$, could be calculated $[Ca^{++}]_i$ from the equation

$$[Ca^{++}]_i = R^2 \times [Ca^{++}]_o$$

The value of $[Cl^-]_i$ was obtained from the equation

$$[Cl^-]_i = \frac{[Cl^-]_o}{R}$$

where R was the measured value of $\frac{[H^+]_i}{[H^+]_o}$.

In this table the values of $[Ca^{++}]_i$ are not very dependable when R is high but values obtained from the last five experiments shown in the table are dependable, becoming more so the smaller the values of R become. The value of P_x is reversed in its direction of action across the membrane at the higher concentrations of $CaCl_2$. The calculated concentration of ions present inside the membrane becomes lower than that necessary to exert the observed osmotic pressure, P_o, against the external solution.

Analysis of these data shows, empirically, that the factor $\frac{E\alpha}{P_x}$ is a constant in those cases (Table II, III and IV) in which the foreign salt concentration (as measured in terms of $[-]_i$, the total negative ion concentration inside the membrane other than the colloid ion itself) in each table of experiments is nearly constant. The factor E is the membrane potential as obtained from the pH measurements, and $P_x = P_c - P_o$ and is a measure of the anomalous pressure found. The factor, α, signifies the degree of ionization of the colloid. Knowing in each case the gram concentration of gum arabic present and its equivalent weight, it is possible to calculate its equivalent concentration. The equivalent concentrations of $[H^+]_i + [Na^+]_i - [Cl^-]_i$ give the total equivalent concentration of positive ions which must be derived from the colloid. This latter value divided by the former gives the percentage ionization, α, of the colloid.

(Continued on Page 285)

TABLE V. [Cl] VARYING. PRESSURE CONSTANT. Na+=CATION.

#	Side of Membrane	Cl conc. eq. x 10^3	Act. coeff. of NaCl γ	γ x Cl conc. eq. x 10^3	Na conc. eq. x 10^3	γ x Na conc. eq. x 10^3	A gm.	F gm.	V cc.	P_o cm. H_2O	EMF mv.	pH	[H+] x 10^7
		In 320 cc. Total soln. (i + o)									H+ ion conc.		
1	i				0.2550	0.2550	.150 .150	8.207 8.235	16.142	131.5	558.6	5.305	49.52
	o								303.56		673.0	7.242	0.5725
2	i	0.032	0.993	0.0318	0.2817	0.2796	.150 .150	8.137 8.125	15.962	130.8	552.7	5.206	62.20
	o								303.74		663.7	7.082	0.8275
3	i	0.096	0.988	0.0948	0.3350	0.3310	.150 .150	7.669 7.601	14.970	131.5	548.2	5.130	74.10
	o								304.73		643.0	6.731	1.857
4	i	0.224	0.983	0.2202	0.4841	0.4760	.175 .175	8.089 7.964	15.703	131.2	526.2	4.767	171.0
	o								303.95		607.7	6.136	7.310
5	i	0.320	0.977	0.3128	0.6066	0.5930	.200 .200	8.626 8.578	16.804	131.0	518.2	4.629	235.0
	o								302.80		593.0	5.887	12.97
6	i	0.960	0.960	0.9220	1.1825	1.1360	.225 .225	6.456 6.493	12.499	130.4	464.8	3.720	1905.0
	o								307.05		508.0	4.450	354.7
7	i	2.240	0.936	2.0970	2.3760	2.2240	.300 .300	5.401 5.367	10.168	130.4	429.2	3.115	7672.0
	o								309.23		456.4	3.577	2648.0
8	i	3.200	0.922	2.9500	3.2610	3.0080	.350 .350	5.077 4.967	9.344	130.1	417.6	2.920	12020.0
	o								309.96		439.8	3.296	5056.0

TABLE V. (Continued)

	E mv.	R	[Na+] x 10⁷	[Cl−] x 10⁷	[HCO_3−] x 10⁷	[T] x 10⁷	[D] x 10⁷	P_c cm. H_2O	$P_c-P_o=P_x$ cm. H_2O	∝	$\frac{E\propto}{P_x}$	$-\log\frac{E\propto}{P_x}$	$-\log[-]_i$	$[-]_i^{.211}$	$\frac{E\propto[-]_i^{.211}}{P_x}$
1 i	114.4	86.50	129800		17.4	129865	126865	320.5	189.0	0.822	0.4975	0.303	5.759	.06240	.03105
1 o			1500		1500	3000									
2 i	110.0	75.15	139800	13.34	11.45	139887	136163	344.1	213.3	0.875	0.4561	0.341	5.605	.06645	.03031
2 o			1861	1003	860	3724									
3 i	94.8	39.90	146300	77.9	14.1	146460	139120	351.5	220.0	0.859	0.3701	0.431	5.036	.08750	.03240
3 o			3670	3110	560	7340									
4 i	81.5	23.40	165950	309		166340	152020	381.9	250.7	0.876	0.2850	0.545	4.510	.11280	.03215
4 o			7090	7230		14320									
5 i	74.4	18.12	177000	568		177800	157720	398.5	267.5	0.873	0.2430	0.614	4.245	.12820	.03114
5 o			9770	10300		20080									
6 i	43.2	5.373	163200	5540		170650	110170	278.4	148.0	0.522	0.1523	0.817	3.246	.20800	.03168
6 o			30360	29770		60480									
7 i	27.2	2.898	190400	23130		221200	85820	217.0	86.6	0.349	0.1097	0.960	2.636	.27920	.03062
7 o			65680	67050		135380									
8 i	22.2	2.378	215300	39530		266850	77200	195.2	65.1	0.295	0.1005	0.998	2.403	.31260	.03141
8 o			90600	94000		189650									

TABLE VI [Cl] VARYING. PRESSURE CONSTANT. Ca^{++} = CATION.

#	Side of Membrane	In 320 cc. Total soln. (i + o) Cl conc. eq. x 10^3	Ca conc. eq. x 10^3	A gm.	F gm.	V cc.	P_o cm. H_2O	H+ ion conc. EMF mv.	pH	$[H^+]$ x 10^7	E mv.	R	$[Cl^-]$ x 10^7	$[Ca^{++}]$ eq. x 10^7	$[Ca^{++}]$ mols. x 10^7	[T] mols. x 10^7	[D] mols. x 10^7	P_c cm. H_2O	$P_c - P_o$ cm. H_2O	α	$\frac{E\alpha}{P_s}$
1	i	0	0.510	.300 .300	5.483 5.416	10.299	128.9	575.0	5.581	26.22	80.8	23.38	0	469500	234750	234800	233500	590.0	461.0	1.000 (assumed)	.1753
	o					309.1		655.8	6 950	1.122			0	859	430	1290					
2	i	0.032	0.333	.180 .180	3.240 3.205	6.085	127.4	552.0	5.192	64.25	75.8	19.18	53.4	411700	205850	205972	204290	516.0	388.6	.8190	.1600
	o					313.5		627.8	6.475	3.350			1023	1119	559	1684					
3	i	0.096	0.590	.300 .300	5.110 5.060	9.570	129.1	550.8	5.171	67.45	69.2	14.87	191.6	697500	348750	349030	344290	870.0	740.9	Impossible	
	o					309.8		620.0	6 343	4.538			2850	3155	1577	4742					
4	i	0.224	0.697	.300 .300	4.892 4.762	9.054	128.2	510.8	4.495	319.8	49.1	6.810	1002	314100	157050	158372	148120	374.2	246.0	.5560	.1110
	o					310.3		559.9	5.328	46.95			6820	6773	3386	10253					
5	i	0.320	0.862	.350 .350	5.368 5.329	9.997	128.2	503.6	4.376	420.5	39.9	4.740	2047	215900	107950	110417	95820	242.2	114.0	.3600	.1260
	o					309.3		543.5	5.052	88.70			9700	9611	4805	14594					
6	i	0.960	1.480	.400 .400	5.011 5.023	9.234	128.0	464.5	3.715	1927	24.0	2.540	10710	170600	85300	97937	56730	143.3	15.3	.2196	.3445
	o					310.0		488.5	4.120	758.2			27220	26462	13231	41209					
7	i	2.240	2.631	.450 .450	4.496 4.455	8.051	127.5	437.5	3.258	5520	16.0	1.867	33320	206500	103250	142090	47310	119.6	—7.9	.1880	—.3810
	o					311.0		453.5	3.529	2957			62200	59243	29621	94778					
8	i	3.200	3.517	.500 .500	4.568 4.489	8.057	128.6	426.7	3.072	8468	13.5	1.695	52330	240500	120250	181048	45500	115.0	—13.6	.1862	—.1850
	o					310.9		440.2	3.301	5000			88700	83700	41850	135550					
9	i	9.600	9.020	.600 .600	3.767 3.785	6.352	127.7	390.7	2.464	34350	5.2	1.228	202400	332950	166475	403225	44165	111.5	—16.2	.1027	—.0329
	o					312.4		395.9	2.553	27980			248700	220720	110360	359060					

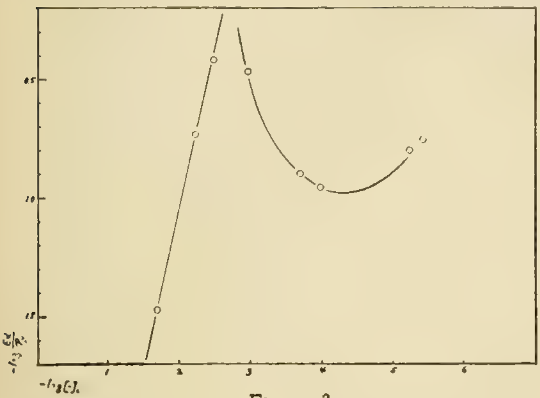

Figure 3.

When, however, the foreign salt concentration, $[-]_i$, is changed radically, as in Table V, the value $\frac{E\,a}{P_x}$ is no longer a constant. Figure 2 shows that the value $\frac{E\,a}{P_x}$ varies with the factor $[-]_i$ in a log-log ratio. Graphic solution of the curve so obtained gives the equation

$$\log \frac{E\,a}{P_x} = -\,0.211 \log\,[-]_i - 1.52$$

Thus, it appears that the value $\frac{E\,a\,[-]_i^{0.211}}{P_x}$ is a constant when, other than the colloid, only monovalent cations and anions are present in the system. This constant does not vary with concentration of colloid, salt, or pH.

When the divalent Ca^{++} ion replaces the monovalent cations in the system, it is seen from Fig. 3 that the linear relationship between $\log \frac{E\,a}{P_x}$ and $\log\,[-]_i$ still holds (at higher concentrations of $CaCl_2$) but that the slope of the curve is much steeper and that as $CaCl_2$ increases in concentration there is a reversal of the effect. At concentrations below this reversal concentration, P_x acts counter to the osmotic gradient, while above this concentration P_x acts in the same direction as the osmotic gradient across the membrane.

While this analysis of these data is empirical and no attempt will be made to devise an explanation for the existence of such a relationship, it may be worth while to point out that the factors, a and $[-]_i$, probably determine the density of charge carried by the colloid particle. It seems possible that the observed anomalies in osmotic pressures of the colloid solutions may through some mechanism be brought about by an interaction of two potentials, one, the electrical potential existing across the membrane, and the other, that determined by the charge carried on the colloid micelle. The reversal of the effect with $CaCl_2$ would seem to indicate that the electrokinetic potential or the sign of charge carried by the colloid is a determining factor in the phenomena.

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Discussion

Dr. Abramson: Did you say that this deviation from the thermodynamically predictable values of osmotic pressures has always been observed, even with proteins?

Dr. Briggs: Loeb found that he could explain the osmotic pressure of casein chloride entirely in terms of the Donnan theory. He could not do so for gelatin salts, however. Others have found that, assuming a definite molecular weight for the colloid, within certain ranges of salt, H^+ ion, and colloid concentrations, the observed osmotic pressure could be completely defined as equal to the sum of the osmotic pressure exerted by the col-

loid micelle, and that exerted by the diffusible ions present in unequal concentrations on the two sides of the membrane. Those who have studied osmotic pressures of colloids of a non-amphoteric nature have, in general, found the observed osmotic pressures to be less than that required from the unequal concentrations across the membrane of the diffusible ions alone.

Dr. Abramson: How do you account for the fact that in some cases the Donnan theory predicts the osmotic pressure, and in other cases it doesn't? You certainly have the charge changing in the case of proteins, and you haven't the difficulty in respect to α. Alpha, in your case, represents a measure of the net charge per average particle weight, unless the solution is mono-disperse, in which case, α would be the net charge per particle. Do you think that the fact that the gum arabic system may be hetero-dispersed might have anything to do with it?

Dr. Briggs: I am not attempting to give an explanation of the anomaly. I am only presenting the facts, first, that so far as the distributions of ions across the membrane is concerned, they are entirely equal to that which is to be expected upon the basis of the Donnan equilibrium theory, as based on measurement of the ratio of distribution of one ionic species (i. e. H^+ ion), and on the assumption of nearly 100% dissociation of all Na and Cl ions present in the system, and second, that the osmotic pressure calculated from such distribution is greater (in cases of monovalent cation salts) than that observed. Whether this may result because the ions, in presence of the colloid, lose some of their osmotic activity, as some suggest, or whether a force, definable in terms of the factors E, α, and $[-]_i$, is working at the membrane, must be left to further experiment to decide. Heterogeneity of the colloid phase, I feel, is of little importance, because in the solution every point of possible ionization is exposed to the solution, i. e., the neutralization equivalent of the arabic acid is independent of the degree of dispersion, at least it is independent of dilution of the solution of the acid. Also, in the analysis of the data, the colloid has been assumed to consist of such large micellae that no osmotic pressure arises from the colloid ion.

Dr. Reiner: Did you determine the activity coefficients of the Cl^- ion in the presence of the gum arabic?

Dr. Briggs: The chloride ion concentration inside of the membrane in these experiments was very low, and no attempt was made to analyse it for total chlorine content at equilibrium. Cl^- ion measurements, made with the silver-silver chloride electrode in these solutions, were very undependable because of the low concentrations. Only in those cases where the $[Cl^-]_i$ became fairly high, i. e., in those cases where total Cl in the system was high, and the ratio of distribution, R, was low, did values of R as determined by the hydrogen ion measurements and those made with the Ag — Ag Cl electrode become equal.

Dr. Mudd: It is not possible to explain your results in terms of an electro-osmotic pressure, acting across the membrane, and giving rise to the anomalous pressure observed?

Dr. Briggs: I must confess that I have thought such an explanation possible. I am, however, convinced that a membrane potential, E, can not function to give rise to electro-osmotic flow of liquid through the pores at equilibrium. While the value of P_x does happen to be definable in terms of this membrane potential, E, and the factors $\alpha + [-]_i$, which could easily be pictured as the determining factors in the electrokinetic potential of the arabic particles, of which the pore walls of the effective membrane could conceivably consist, I think that some other mechanism must be sought out to explain the anomalies observed.

OSMOTIC PRESSURES IN RELATION TO PERMEABILITY IN LARGE PLANT CELLS AND IN MODELS

W. J. V. Osterhout

Plant cells are especially suited to the studies which here concern us[1]. As a rule they consist of a thin protoplasmic sack with cell sap inside, and a cell wall outside. When the osmotic pressure in the sap is higher than in the external solution, water enters and distends the protoplasmic sack until it presses against the cell wall (somewhat as the inner tire of an automobile is pressed against the outer shell). When the osmotic pressure in external solution becomes greater than in the sap, water goes out, and the protoplasmic sack shrinks away from the cell wall: this is called plasmolysis. This process affords a means of measuring the osmotic pressure of the sap, by finding what osmotic pressure in the external solution causes water to move out.

To be a perfect osmometer, the protoplasm should be permeable only to water and not to dissolved substances. This is never the case, but it is approximated with certain substances which enter very slowly. By ascertaining how rapidly various substances enter, we can gain an idea of the nature of the protoplasmic surface. Overton formulated the hypothesis that the surface is lipoidal, and that only those substances which are soluble in lipoid can enter. We can agree to this if we widen the conception by saying that the surface consists of a non-aqueous layer, and if we also take into account the relation between this layer and the aqueous protoplasm, or sap, as formulated by Irwin in the hypothesis of multiple partition coefficients.

This hypothesis has furnished a useful means of approach to the central problem which here concerns us, namely, how can the cell absorb water and increase its volume indefinitely, and still maintain a higher osmotic pressure than exists in the surrounding solution? If it did this by manufacturng substances like sugar, the problem would be simple. But, in such a cell as *Valonia*, we find osmotic pressure in the sap to be almost wholly due to KCl and NaCl. The concentration of KCl is about forty times as great as in the sea water outside. By what mechanism is this maintained?

Since the sap is more acid than the sea water, it seems possible that potassium enters chiefly as KOH which becomes neutralized in the sap. This may come about in much the same manner as in certain artificial cell models[1]. In these, potassium passes from an aqueous alkaline solution, *A*, through a nonaqueous layer *B* (representing the protoplasm), into an aqueous phase *C* (representing the cell sap). At the start, *C* consists of distilled water: CO_2 is bubbled through *C*, during the experiment, to imitate the production of CO_2 in the cell. In *B* we place a mixture of guaiacol and *p*-cresol: these may be collectively designated as H*G*.

When we place KOH in *A*, it at once reacts to form K*G*, according to the scheme

$$KOH + HG = KG + HOH.$$

On arriving at *C*, K*G* reacts with CO_2 thus

$$KG + H_2CO_3 = KHCO_3 + HG.$$

This process tends to go on as long as the ionic activity product (K) (OH) is greater outside than inside. Since the concentration of KOH in *C* is kept constant, and since the bubbling of CO_2 keeps down the concentration of OH in *C*, potassium continues to enter *C*, until its concentration is many times as great as in *A*. The osmotic pressure in *C* exceeds that in *A*, so that water moves from *A* to *C*. Eventually a steady state is reached, in which water and electrolyte enter *C* in a fixed ratio, so that the volume in *C* increases, while its composition remains approximately constant, with a higher osmotic pressure than in *A*. Something like this seems to take place in living cells.

It is of interest to note that in this model we derive no energy from the formation of CO_2, but employ CO_2 after it has been formed, using what is ordinarily regarded as a waste product of the living cell.

We find that the order of penetration is the same as in *Valonia*, *i. e.* K > Na > Ca > Mg. To explain the difference in rates of entrance, *e. g.* between those of sodium and potassium, we must consider conditions in *B*, where the rate of diffusion is so slow that it controls the whole process of penetration. Although the diffusion constants of the two salts in *B* are about the same, the partition coefficient of the potassium salt is so much higher that its concentration gradient in *B* is much steeper, in consequence, more potassium than sodium moves through *B*, in unit time[2].

1. Large multinucleate cells such as those of Valonia are especially suitable for these studies. For the literature up to July, 1931, dealing with such cells see Osterhout, W. J. V., Biol. Rev., 1931, 6, 369.

1. Osterhout, W. J. V., and Stanley, W. M., J. Gen. Physiol., 1931-32, 15, 667.

2. Osterhout, W. J. V., J. Gen. Physiol., 1932-33, 16, 529.

There is an important difference between the model and *Valonia*, since in *Valonia* potassium accumulates as KCl: this might be due to the fact that as bicarbonate increases in the sap, it tends to go out in exchange for chloride which comes in from the sea water. It should be remembered, however, that in many cells[1] where oxalic, malic, citric, tartaric and other acids are produced in considerable quantity, potassium may accumulate as the salts of these acids. This would be quite comparable to the accumulation of $KHCO_3$ in the model. We have set up models with formic and citric acids in place of carbonic, and have found that potassium readily accumulates as formate and citrate.

It is worth while to consider the manner in which electrolytes pass through the non-aqueous layer. In the model, KOH reacts to form K*G* in *B*, and this reacts to form $KHCO_3$ in *C*. The net result is that *A* loses a potassium ion, and *C* loses a hydrogen ion, and from a thermodynamic standpoint it amounts to an ionic exchange, *i. e.* K^+ passes from *A* to *C*, and H^+ passes from *C* to *A*. But when we consider rates, we see that this picture is wrong. On the basis of ionic exchange, it would make no difference whether we placed in *A* 0.01 M K*G* or 0.01 M KCl. But from a kinetic standpoint, it makes an enormous difference, since K*G* penetrates very much faster than KCl, because its partition coefficient, and consequently its concentration gradient, in *B* is so much greater. The relatively rapid movement of K*G* in *B* is almost wholly in molecular form, since it is a very weak electrolyte in *B*.

The model illustrates a possible mechanism by which the living cell can absorb water and expand indefinitely, and yet maintain a higher osmotic pressure than exists in the external solution. To maintain such a difference evidently requires an expenditure of energy. This takes place in the model in an interesting way which is best seen when we substitute HCl for CO_2, and leave the system to itself, instead of renewing certain substances.

We then have KOH on one side of the non-aqueous layer, and HCl on the other. They tend to mix by passing through this layer, thus forming KCl in *A* and in *C*. But KOH moves so much faster that most of the KCl is formed in *C*, where the concentration of potassium becomes much greater than in *A*. This, however, is only temporary, for when the system comes to equilibrium the composition of *A* and *C* will be identical, since all the substances present are able to move through the non-aqueous layer.

If we designate the difference in thermodynamic potential between KOH in *A* and *C* as Δ_{KOH} it is evident that Δ_{KOH} steadily falls, and eventually equals zero. This is also true of Δ_{HCl}.

The case is quite different for the accumulating substance[1], KCl. We find that Δ_{KCl}, starting from zero, rises to a high value, and then falls to zero at equilibrium.

In a model with KOH and HCl constantly renewed[2], Δ_{KCl} would rise steadily until it reached an approximately constant value. This constant value would be maintained in the same way as that of $KHCO_3$ in the model previously mentioned.

The same principles may apply to living cells, particularly where potassium accumulates, combined with organic acids (e. g. as potassium oxalate). Such cells closely resemble the model.

These experiments serve to illustrate some of the methods and conceptions which have been found useful in a field which has many attractive problems.

Discussion

Dr. Chambers: Do either narcotics or oxidation-inhibiting agents interfere with the selective passage of cations into the vacuole of *Valonia*? And do they interfere with the working of the model?

Would it be possible to stop the liberation of CO_2 into the vacuole and to determine whether this interferes with the accumulation of HCl in the living cell?

If the continual evolution of CO_2 on one side of the membrane is necessary for the passage of materials through the membrane, how would you explain conditions of plant cells with chloroplasts in bright sunlight when there exists a CO_2 lack in the protoplasm to the extent that CO_2 is being consumed from without?

Also, what is the source of carbon with which the cell must be continually supplied in order to produce the CO_2? The supply would have to be enormous.

Dr. Osterhout: Experiments with narcotics have not yet been made with *Valonia* but cells were placed in a refrigerator to check the production of CO_2. We might then expect the entrance of potassium to fall off more than that of sodium because as the pH value of the sap rises the difference between the ionic product inside and outside approaches zero much more rapidly in the

1. Czapek, F., Biochemie der Pflanzen, Jena, Gustav Fischer, 3rd edition, 1922-25. For potassium oxalate see Vol. 3 p. 69: for other organic acids consult the following pages. See also Evans, H., Biol. Rev., 1932, 7, 181.

1. Unpublished results.

2. Here the movement of HCl would have comparatively little effect on the composition of A.

case of potassium. For example, if we raise the pH of the sap to 6.3 the ionic activity product (K) (OH) inside becomes approximately equal to that outside and we should, therefore, expect the entrance of potassium to stop but sodium should continue to enter because the ionic activity product (Na) (OH) remains much greater outside.

We found that this actually happened when the pH of the sap was raised by the entrance of NH_3 and we surmised that in the refrigerator something of the same sort occurred but that the entrance of potassium was merely slowed down instead of being stopped altogether. At any rate in the refrigerator the ratio $K \div Na$ in the sap fell off, as would be expected. But unfortunately no determinations of the pH value of the sap were made in the refrigerator experiment.

In daylight the cells quickly raise the pH of the sea water just outside the protoplasm from about 8 to about 9.6 but the pH of the sap remains practically unchanged. Hence the difference of pH between inside and outside increases in the light, and the entrance of potassium and sodium is hastened.

The cell maintains the pH of the sap at a much lower value than that of sea water. It averages about 5.8, that of sea water being about 8.0. The difference seems to be due mostly to CO_2 since the sap contains very little organic matter (about 1.4 parts per thousand).

In the model there is no oxidation. We make no use of the energy set free in producing CO_2 but start with it already formed. Hence experiments with narcotics have no bearing on the operation of the model. But we can slow down the rate of bubbling of CO_2 and the entrance of electrolytes then slows down.

Applying substances intended to lessen production of CO_2 might have a variety of effects. For example, in applying KCN to *Valonia* we should have to do with the entrance of KOH and of HCN and probably with changes in the pH of the sap. Injury might result in increasing permeability so that potassium would come out and sodium go in. Experiments of this sort may, therefore, be difficult to interpret.

Dr. Fricke: What is the water content of the guaiacol?

Dr. Osterhout: Dr. Shedlovsky found that 100 gm. guaiacol dissolves 4.63 grams of water.

Dr. Fricke: Would you discuss the actual use which the plant can make of these various differences (in osmotic pressure, electric potential, chemical composition) between the plant interior and the surrounding fluid?

Dr. Osterhout: The difference between the inside and outside is so characteristic of living things that it seems essential to the conception of life. A simple experimental test is to place *Valonia* in its own sap in which it quickly dies.

To be more specific the higher osmotic pressure inside the cell causes water to enter and thus brings about growth. Even when the cell is not actually expanding the higher osmotic pressure inside keeps the cell wall from collapsing and maintains the form of the cell. In the higher plants the lack of such pressure produces wilting.

The potential difference across the protoplasm is a necessary condition for action currents. It may also cause movements of water and of electrolytes where local differences of potential permit a flow of current. To what extent such current flow may be of use in correlating the activities of various parts of the organism is an open question.

The difference in chemical composition between the protoplasm and the outside is, of course, of fundamental importance, but that between the vacuole and the outside depends on circumstances. In *Halicystis* the difference is not great aside from the fact that the sap has a higher acidity (which is probably necessary for the entrance of electrolytes), more organic matter and less oxygen: part of the time at least the content of free CO_2 is higher. In most organisms there are other differences to which I am unable to assign any teleological significance.

Dr. Fricke: Can you say anything about the reason why potassium should go through more than sodium?

Dr. Osterhout: The essential reason is that potassium has the higher partition coefficient.

Dr. Fricke: As soon as you stop carbon dioxide production does the process immediately reverse and the potassium begin to diffuse out?

Dr. Osterhout: When the bubbling of CO_2 ceases in the model potassium begins to move out, but we have not followed the process to equilibrium since it takes a long time.

Dr. Fricke: If *Valonia* is not completely impermeable to sodium would you not expect that sodium would in time come into equilibrium inside and outside? Or is such an equilibrum not reached within the life of the plant? The red corpuscle presents a similar difficulty.

Dr. Osterhout: No such equilibrium is reached in *Valonia*. Ordinary equilibrium would mean identity inside and outside. Donnan equilibrium would mean that the activity of the hydrogen ion inside should stand in the same relation to that outside as in the case of potassium and sodium. This is very far from being true.

What we apparently have in the model and in *Valonia* is a steady state in which water and electrolyte enter in a fixed ratio so that as the volume

increases the composition remains approximately constant.

Dr. Abramson: Is there evidence that this model proposed for *Valonia* may also be applied to the red blood cell which has in some cases been considered as in thermodynamic equilibrium?

Dr. Fricke: The red cell comes into thermodynamic equilibrium if it is actually impermeable to sodium and potassium. But that is the question. Is there anything that is actually impermeable?

Dr. Cohen: If you asked the proponents of the viewpoint that there was thermodynamic equilibrium, I think they would agree that absolute impermeability to sodium was never intended.

Dr. Osterhout: The scheme proposed for *Valonia* is one for actively growing cells and hence does not seem to apply to the erythrocyte. It may be said, however, that in the case of potassium in *Valonia* two processes go on simultaneously, (1) the entrance of potassium, probably as KOH, and (2) the exit of potassium, as KCl. As the cell grows older and produces less CO_2 the first process will fall off, but the second will not. When the exit becomes equal to the entrance we might have a steady state without growth, but this would be very different from equilibrium.

Dr. Fricke: Is the membrane distinct from the protoplasm, or is it just the surface of the protoplasm in this particular case?

Dr. Chambers: It is the surface of the protoplasm.

Dr. Fricke: Is there direct evidence that it is?

Dr. Chambers: There is indirect evidence. Non-penetrating dyes injected into the interior do not pass out, or if you put them on the outside they never go in, so there must be something on the surface that keeps them from going in, or out.

Dr. Mudd: Would it not be interesting to inject with a micropipette droplets of various oils and aqueous solutions between the outer wall and the protoplasm of *Valonia* and other plant cells? Direct evidence concerning the wetting properties of the protoplasmic surface might thus be obtained.

Dr. Chambers: Oil drops can be applied to the external surface of plasmolysed protoplasts. This has been done with the epidermal cells of the onion bulb scale. Plasmolysis causes the protoplast to shrink and draw away from the cellulose wall leaving a space between the wall and the protoplast. When a strip of plasmolysed tissue is chopped into bits at right angles to the long axes of the cells one may find many cells on the border of the cut with thin end walls cut off but with their protoplasts still intact.

If a relatively large drop of olive oil is applied to the exposed surface of the protoplast the oil snaps on and starts to engulf it. Before the process is carried to completion the protoplast bursts.

If the oil drop is very small you get the reverse. The droplet is engulfed by the protoplast.

Dr. Mudd: Is the oil of the same composition in both cases?

Dr. Chambers: The oil applied in both cases was the same.

Dr. Mudd: For the protoplast to engulf the oil, the tension at the oil-medium interface must be greater than the sum of the tensions at the oil-protoplast and the protoplast-medium interfaces. For the oil to engulf the protoplast, the tension at the protoplast-medium interface must be greater than the sum of the tensions at the oil-medium and oil-protoplast interfaces. Both conditions can not obtain for the same system.

Dr. Chambers: For the small drop of oil, material dissolves in it, so that the surface forces change. In the large oil drop the surface forces remain of one order.

Dr. Osterhout: Would it be possible to apply the drops of oil to the inner surface of the protoplasm which is in contact with the vacuole? When the outer surface is drawn away from the cell wall by plasmolysis it tends to secrete a new cell wall and this may possibly begin as soon as plasmolysis takes place. No such formation of cell wall occurs on the surface in contact with the vacuole, so that here, if anywhere, we might expect naked protoplasm.

OSMOTIC BEHAVIOR OF RED CELLS. I.

Eric Ponder

When one succeeds, by using some well known physical principle, in reducing to order some complex biological phenomenon, one can sit down to write a lecture about it with all the élan which properly accompanies the production of a rabbit from a hat. But when, by closely examining a supposedly simple biological phenomenon, one finds that the physical principles which have always been supposed to apply to it do not do so in fact, and that the more thorough one's examination, the further do the results depart from expectation, one is not quite so comfortable. Under such circumstances there is nothing to be done except to give an account of one's experiments, of why they were carried out, and of the results which emerge. This is what I propose to do in speaking about the osmotic behavior of red cells, and if this lecture seems to be too personal an account of matters which appear more specific than general, I must excuse myself by saying that the erythrocyte has not been disposed to behave itself as accountably as might be wished.

Some years ago, I and my collaborators, after having measured red cell diameter and thickness almost *ad nauseam,* decided to make some really good measurements of red cell volume. Quite a number of methods presented themselves, e. g., refractometric methods, methods employing various kinds of chemical analyses, viscosity methods, and so on, but all of these we rejected after examination. Only two methods seemed promising, the haematocrite method, and a modification of an old colorimetric method of Stewart(1)(2)(3). This last I shall describe in some detail, and I shall refer to the haematocrite method in its proper place.

The principle of the colorimetric method consists in mixing a known volume of a solution of the animal's own haemoglobin, dissolved in its own plasma, with a known volume of the animal's whole blood, determining the extent to which the pigment has been diluted by the plasma contained in the whole blood, and then calculating the volume of such plasma present, and thence the volume of the cells. Division of the latter figure by the number of cells present, as determined by a careful count, gives the mean volume of the single cell. If properly carried out, this method is very accurate indeed, and will give the mean volume of the red cells of such an animal as the rabbit (volume about 60 μ^3) to within about $\pm 1\mu^3$. Its use, however, is rather limited, as will be seen below, for while it is excellent for finding volumes in plasma, in isotonic solutions, and in certain hypertonic and hypotonic solutions, technical difficulties prevent its use for finding volumes in very hypotonic solutions, and it is in these, of course, that we are most interested. The method, nevertheless, is a kind of "standard method", to which others may be compared.

Using this procedure, we first determined the mean cell volumes for a number of animals, and then went on to examine the alterations in cell volume in solutions of different tonicities, for such knowledge is clearly indispensable in connection with problems relating to red cell permeability in general(4). It is true that the relations between tonicity and red cell volume had been studied at least since the time of Hamburger's investigations, but we soon appreciated the fact that the method almost invariably used for measuring cell volume, the haematocrite method, is subject to errors about as great as many of the changes which have to be detected. This point I shall return to directly; in the meantime it is sufficient to say that we began by trying to measure the mean volume of rabbit red cells suspended in mixtures of plasma and NaCl of tonicities from about the equivalent of 1.6 p. c. NaCl (grams per 100 grams water) to about the equivalent of 0.8 p. c. NaCl, and in mixtures of plasma and KCl and of plasma and glucose, covering about the same tonicity range. At this point I may remind you that rabbit's plasma, which is presumably isotonic with the interior of the rabbit erythrocyte, has a tonicity corresponding to about 1.1 p. c. NaCl as determined by freezing point depression measurements, i. e., a tonicity roughly in the middle of the selected range. I ought also to emphasize that the colorimetric method involves the addition of haemoglobin in plasma to whole blood, and that the systems therefore contain a very considerable amount of plasma, although the latter is rendered hypertonic by the addition of hypertonic NaCl, KCl, or glucose, or hypotonic, by the addition of hypotonic NaCl, KCl, or glucose, as the case may be.

At first we adopted the plan of measuring the volume of the cells in all the systems of differing tonicity in one experiment, but the results turned out to be exceedingly irregular. For example, in one experiment we would obtain the following values:

Tonicity	**0.8**	**0.9**	**1.0**	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	**Plasma**
Vol., μ^3	**60.7**	**58.6**	**57.1**	**55.9**	**54.2**	**53.1**	**51.9**	**51.6**	**53.8**

the "normal volume" of the cells (i. e., their volume when suspended in plasma) being maintained in a mixture of plasma and NaCl of an equivalent tonicity of about 1.2 p. c. NaCl, while in hypo-

tonic solutions there was swelling and in hypertonic solutions shrinking. In another experiment, however, we would get:

Tonicity	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	Plasma
Vol., μ^3	77.1	75.5	73.5	71.9	70.4	69.9	65.1	63.8	63.7

all the solutions now bringing about swelling.

Contradictory experiments of this kind soon showed us that some factor was being neglected, and this turned out, as so often happens, to be a time factor, the volume of the cells depending not only on the tonicity of the environment, but also on the length of time during which the cell had been exposed to the particular hypertonic or hypotonic medium surrounding it. Once this was taken into account, all the irregularities disappeared, and we began to get consistent results of the kind shown in the following table, in which the volume of rabbit red cells, exposed to NaCl-plasma mixtures of various tonicities, were measured colorimetrically after various intervals of time. All the volumes are expressed as percentages of the "normal volume" found for the cells suspended in plasma.

Final tonicity g. NaCl per 100 g. water	Cell volume 5 min.	30 min.	1 hr.	2 hr.	3 hr.
0.81	104	105	105	109	—
0.91	102	105	105	105	105
1.02	102	100	104	101	—
1.12	99	100	101	100	100
1.22	103	104	—	105	—
1.32	97	100	102	104	—
1.42	102	103	106	106	108
1.52	104	109	110	110	111

The most striking point about these results is the swelling, instead of the shrinking, which occurs in hypertonic solutions, although with considerable irregularity, even considering that the method has an error of at least 2 p. c. attached. This swelling, furthermore, often increases with time, as the figures for the tonicities 1.32 and 1.42 illustrate very well. There is, of course, one particular concentration of NaCl, in this case 1.12 p. c., in which the "normal cell volume" is maintained, but for concentrations both greater than this and less than this, the behavior of the cell is quite anomalous, for in the former there is often swelling instead of the expected shrinking, and in the latter, although the figures of this table do not show it unless calculations are made, the swelling is considerably less than one would expect it to be. One requires some explanation for these anomalies, especially as exactly the same sort of behavior is met with in plasma-KCl and plasma-glucose systems; clearly we are not dealing with a "simple" or "perfect" osmometer, which, being impermeable to cations and other osmotically active substances, loses or gains water only, and thus changes in volume as required by the Marriotte law in its simplest form.

Now this conclusion, obvious though it is, is not far removed from physiological heresy, for if there is any one point which is generally accepted about the red cell, it is that it is impermeable to cations, the principal osmotically active substances which it contains. The validity of many of the calculations of Van Slyke(5) (6), L. J. Henderson(7), Peters, and many other investigators depend entirely on the existence of this impermeability, which is accepted by almost everybody, although Hamburger and one or two others have held that there is an Na-K exchange across the membrane, and have supplied evidence for it which, in my opinion, has been disregarded rather than disapproved in its entirety. One must bear in mind, nevertheless, that Henderson, Van Slyke, and others who work along the same lines, are concerned with the behavior of the erythrocyte in normal plasma, and not with the volume changes which it undergoes in grossly hypertonic or hypotonic media. Within the "physiological range" its behavior may quite well be much simpler than its anomalous behavior outside that range, or, if not actually different, at least not sufficiently anomalous to attract attention. The colorimetric measurements of volume show, indeed, that in a NaCl-plasma mixture of about the same freezing point as that of rabbit plasma (equivalent to about 1.1 p. c. NaCl), the rabbit red cell maintains its normal volume more or less indefinitely, i. e., it behaves as if it were impermeable to cations, although in hypertonic and hypotonic solutions, (as we shall see even more clearly below) it behaves quite otherwise.

One can thus easily enough wriggle out of the apparent disagreement between the results of the experiments in hypertonic and hypotonic solutions and the accepted impermeability to osmotically active substances, for the latter can be put down as being a property of the red cell membrane when in its normal environment of isotonic plasma, although not necessarily as a property of the cell when in a non-physiological medium. Unfortunately, however, one cannot evade with equal facility the very definite claim that the erythrocyte, when surrounded by hypertonic or hypotonic media, exchanges water according to the Boyle-Marriotte law, and, in doing so, still behaves as a "perfect osmometer" impermeable to cations and other osmotically active substances. This latter claim is not put forward as a postulate, as is the idea of impermeability in isotonic plasma (which one can scarcely, in the nature of things, prove or disprove), but as an experimental fact(8) (9),

and so I shall be required to describe briefly the kind of experiments on which it is based.

These experiments consist almost entirely of determinations of red cell volume by the haematocrite method, in which a volume of red cells with the medium in which they are suspended is placed in a narrow capillary and spun at high speeds until the column of packed cells either shows no further decrease in length on further spinning, or shows a kind of translucence known as Koeppe's criterion. If the same number of cells is suspended in solutions of different tonicity. (e. g., plasma, diluted plasma, etc.) the percentage swelling in each of the hypotonic solutions can be obtained by dividing the length of the column for each of the hypotonic solutions by the length of the column for the cells in plasma, the volume of the latter in this way being regarded as 100 p. c., and all other volumes as percentages of it. And similarly for hypertonic solutions.

Now let us imagine that we are dealing with cells which contain 67 p. c. of their volume (the usual figure) as water with osmotically active substances dissolved in it, the tonicity of the cell interior being the same as that of plasma, which we shall call 1.0. Then if the cells are placed in media containing plasma, plasma diluted with water so as to give a tonicity of 0.9, 0.8, 0.7, etc., and if the cells reach equilibrium with the hypotonic solutions by a water exchange *alone*, we must have the following volumes attained at equilibrium, the volume of the cells in plasma being denoted by 100, as before:

Plasma	Diluted plasma				
= 1.0	0.9	0.8	0.7	0.6	0.5
100	107	117	129	144	167

Ultimately, of course, the cell attains a certain "critical volume" at which it can swell no further without haemolysing. This is the result which is consistent with the cell's being a "perfect osmometer", and swelling as a result of water exchange alone.

Some investigations by the haematocrite method have shown that this kind of result is obtained, but others have shown that it is not, and that the swelling is always considerably less than that demanded by the Boyle-Marriotte law. Under any circumstances, the results are irregular and variable. The difficulty is one which is inherent in the haematocrite method itself, for the result one obtains depends very largely on the rate of spinning which one uses, and the "right rate" may be, and is usually, quite different for cells in plasma and for cells in a hypotonic medium. In a lecture of this sort any consideration of the fallacies of the method, or even on account of the divergent results which it has given in the hands of different investigators, would be out of place, but we can summarize the situation, so far as haematocrite determinations go, by saying that the swelling and shrinkage of red cells is usually, although not always reported to be less than it should be. This is the same sort of result as emerges from my own colorimetric determinations. The latter, however, are obtainable only over a very limited range, and so we have to look for another method of determining red cell volume, and it is necessary that such a method shall be able to give values for volume even in very hypotonic solutions.

Provided that a certain amount of care is used in applying it, the ideal method for measuring volume is a diffraction method in which the cells are converted into spheres without any change in their volume, and in which the mean volume is found from the diffractometric measurement of their mean radius[10]. To turn the normal discoidal cells into spheres, all that is necessary is to suspend them in a hypertonic, isotonic, or hypotonic saline, and then put them between a slide and a closely applied coverglass[11]. For some reason, at present obscure, they then become perfectly spherical, and it can be shown in various ways that this change of shape does not involve a change in volume. The diffratometric measurement of volume is very accurate, and, by making volume measurements for cells in plasma or in a saline isotonic with it, for cells in hypertonic solutions, and for cells in hypotonic solutions, one can show quite readily that the Boyle-Mariotte law roes not apply at all. In hypertonic solutions one gets the same rather irregular shrinkage as the colorimetric method shows, or even sometimes swelling. In isotonic solutions (about 1.1 p. c. NaCl) one gets a maintenance of the normal volume, and in hypotonic solutions one gets much less swelling than the Mariotte law demands, although the results are perfectly regular, and the "swelling curves" obtained by plotting relative cell volume against tonicity are perfectly smooth up to the point where lysis begins. On the basis of these experiments, together with the restricted results obtained colorimetrically and the rather unreliable results obtained by the use of high speed haematocrites, I have no hesitation in saying that the erythrocyte does not swell, or shrink, as if it were a "perfect osmometer" reaching equilibrium by water exchange alone. And at this stage I want to remark that this is a conclusion of fact, and that it does not necessarily involve the acceptance of any particular theory which may be put forward to account for it, especially the theory which I shall put forward directly.

If we admit, then, that the red cell does not behave as the Mariotte law requires, what explanation can we give for the divergence? Quite a number of observers have recognized, to a

greater or lesser extent, that the divergence exists, and it will be simplest if I tabulate the explanations which have been offered.

1. It is conceivable that water should enter the cell from a hypotonic medium, that the cell should swell, but that its expected swelling should be prevented by its membrane being stretched and then exerting a "back pressure" equal to the difference between the osmotic pressure of the interior and the surrounding medium at equilibrium. In the case of the red cell, this explanation can scarcely be countenanced, for the membrane is less than 1μ thick, and could not stretch to the required extent within its elastic limits.

2. It is possible that either the cell contents or the medium which bathes the cell might undergo dissociation in a very anomalous manner, so that osmotic equilibria, as calculated in the usual way, would not be equilibria at all. Ege has considered this possibility, and has rejected it. One must express one's self guardedly nevertheless, for osmotic equilibria in systems containing large amounts of protein on one side of a membrane are sometimes very anomalous indeed. Considering, however, that the electrolyte concentrations are relatively high, and that the range of tonicity over which we work is really exceedingly small, I doubt if anomalous dissociation could account for the relatively large divergences observed.

3. In a similar way we have to consider the possibility that the red cell interior has initially an osmotic pressure different from that of the surrounding plasma. This used to be thought to be the case, but recent investigations lead to the far more likely conclusion, viz., that the interior of the cell is initially in equilibrium with its surroundings.

4. When it was first noticed that the swelling which occurs in hypotonic solutions is less than that expected on the basis of Marriotte's law, various observers introduced the idea of "bound water" rather than abandon the idea that the cell is a perfect osmometer. Thus, if the cell actually contains, as shown by analysis, 67 p.c. of water by volume, some of it was supposed to be "osmotically inactive" or "bound," and the rest "free." If such a state of affairs were actually to exist, the cell would, of course, reach equilibrium with a hypotonic medium by taking in about half as much water as it would have to do if all its water were "free," and so the small volumes met with in hypotonic solutions would be accounted for without abandoning the idea of impermeability to cations and other osmotically active substances. This "bound water" idea has had quite a vogue, parallelled by the idea of "bound water" in muscle and in other cells which do not swell "as they ought to do" in hypotonic solutions. No one, of course, has ever demonstrated the existence of this bound water by independent means, and recently Hill has shown that, even if it exists at all, it makes up only about 5 p.c. of the total water present in the cell[12]. There has nevertheless developed quite an extensive literature regarding the purely imaginary substance, because of the principle of *sancta simplicitas,* I imagine, rather than any other.

5. Although most physiologists have considered the erythrocyte as being impermeable to cations, etc., this assumption and its consequences have usually been confined to the erythrocyte when bathed in plasma, and when the cell is suspended in hypotonic media there is, as I have already remarked, no reason to abide by it. In fact, there is plenty of evidence that under such circumstances the cell actually loses cations (mainly K) into a hypotonic environment, and the existence of the cations lost has been demonstrated in the suspension medium by chemical analysis by Kerr [13] [14] [15], Neubauer and Breslin[16], and others. When the suspension medium is glucose, it is universally admitted that there is a loss of cations from the cell, and this can be shown conclusively by conductivity measurements. In fact, there is abundant evidence to show that while the cell may be a more or less perfect osmometer when in plasma, in which it is not called upon to be much of an osmomter at all, it is a very imperfect one when the medium is NaCl, KCl, or glucose, especially if the solutions of these substances are hypotonic.

I now propose to put together two facts: (1) If the red cell were a perfect osmometer, it would swell in hypotonic solutions according to a regular swelling curve, calculable from Marriotte's law, and (2) the cell in fact swells in such a way as to give a regular swelling curve, but a different one from that calculable from Marriotte's law. The fact that both the "theoretical" and the observed swelling curves are regular and of the same general form does not appear to have been commented upon, and so we shall examine the relation between the two curves more closely. At once we may observe that the difference between two curves of similar and regular form must itself be describable in simple, if empirical, terms.

To make things as simple as possible, I propose to use the fiction of "bound water." We shall think of the cell as a perfect osmometer. If all its water were "free" it would swell according to Marriotte's law. In fact, it swells less, so we shall suppose that some of the water is "bound." Now let us denote the tonicity of the medium by T, and let us put the tonicity of the cell interior as equivalent to a 1.1 p.c. NaCl. Let us also use v to mean the percentage increase in cell volume when the cell reaches equilibrium with a hypo-

tonic solution. Then, if everything is expressed in the proper units, we have as an equilibrium condition,

$$\frac{1.1\,Q_2}{Q_2 + v} = \frac{T}{100 \quad v} \qquad \ldots . (1)$$

where Q_2 is the quantity of free water present in the cell. Let Q_1 be the total quantity present; then let us use the fraction

$$R = 1000\ Q_2/Q_1 \qquad \ldots . (2)$$

as a measure of the fraction of the total water which is free, or, alternately, there being no bound water in fact, of the fallaciousness of the idea that we are dealing with a simple osmometer. Then if $R = 1.0$, our osmometer is perfect; in fact, however, the values of v found experimentally are always such as to give a value of R of about 0.5, or even, in the case where glucose is the suspension medium, of 0.3, and so we require to find a reason, in quantitative terms, why this should be so.

There are many conceivable explanations, but the most likely is this. Let us imagine that the cell, when suspended in a hypotonic medium, is not wholly impermeable to osmotically active substances, but that as it gains water it loses a quantity of osmotically active substance x, thereby reaching equilibrium. Then we must have

$$\frac{1.1\,Q_1 - x}{Q_1 + v} = \frac{T}{100 - v} \qquad \ldots . (3)$$

x being, in the units used, a quantity of osmotically active substance in grams lost by a litre of cells, and expressed as equivalent to a NaCl solution of the same freezing point. The units are troublesome, as they always are in equations involving tonicities, but if we express x as a function of the original amount of osmotically active substance in the cell and call it X, we arrive at the curious relation

$$dX/dT = \text{const.} \propto 1/R \qquad \ldots . (4)$$

Equal steps of tonicity thus result in the loss of equal amounts of osmotically active substances from the cell, the smaller the value of R, the greater being the loss per unit step. As an instance, take the case where $R = 0.5$, i. e., where the cells swell as if only half their water were "free," and let us take the tonicity of the cell interior as 1.0. Then as we pass one tonicity to another, immersing the cells in 1.0 p.c. NaCl, 0.9 p.c. NaCl, 0.8 p.c. NaCl, and so on, the quantity of osmotically active substances lost increases in the following way:

Tonicity, g.NaCl p.c.	**1.0**	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**	**0.4**
X, p.c. original NaCl lost ..	**0**	**5**	**10**	**20**	**30**	**40**	**50**

Equal steps in tonicity thus correspond to losses of equal amounts of osmotically active substances.

The idea of the loss of cations, etc., from erythrocytes in hypotonic media is not in itself at all new, as I have already remarked; the interesting point, however, is the regular way in which the loss appears to occur, for the observed swelling curve differs from the theoretical one in just such a way as one would expect if some constant factor were operating to bring about the divergence. The presence of "bound water" would do as a factor, but this substance does not exist in appreciable quantities. The loss of cations, etc., is a sufficient explanation, especially as we have independent evidence that it occurs, but it is not the only possible explanation, nor it is necessarily completely adequate in a quantitative sense, for a cation loss, together with other modifying factors which operate as regular functions of tonicity might be necessary to account for the phenomenon fully. The important point, to my mind, is that all these factors do in fact seem to operate as regular functions of tonicity, and, indeed, if expression (4) is correct, as linear functions, at least when considered in the aggregate, and that the red cell, although it does not behave as a perfect osmometer in the sense of Marriotte's law, behaves in a very orderly fashion according to a law of its own.

So far I have been speaking of systems containing red cells in NaCl, KCl, or glucose of different tonicities, plasma being absent in all those cases in which the measurements were made diffractometrically, for red cells will not become spherical in the presence of plasma. Plasma is certainly present in those systems in which the volume measurements were made colorimetrically, but, as I have said already, this method covers too small a range to provide us with much information regarding swelling in hypotonic solutions. The swelling curves and the expressions which apply to them (expressions 1, 2, 3 and 4) are thus based on systems in which the cells are suspended in plasma-free media, and we have now to take the next step and see what happens when the cells are suspended in media containing plasma which has been rendered hypotonic by the addition of water. This step is important, for it may be laid down as a principle that no red cell (and probably no other vertebrate cell) is "normal" unless it is suspended in the plasma of the animal from which it is derived. In the case of the erythrocyte, its form and metabolism are both dependent on its environment, and cells suspended in saline, however well buffered or balanced, are different in many respects from the same cells in plasma. So far as their behaviour in hypotonic solutions is concerned, cells in NaCl, KCl, etc., might be very different from cells in

plasma, for there are at least three factors which might bring about a difference.

1. The volumes attained in hypotonic NaCl, KCl, and glucose are found diffractometrically, the cells being converted into spheres by being placed between a slide and a coverglass. The measurements of volume are themselves reliable, but the objection can always be raised that the same kind of unknown forces which change the form of the cell may also alter its permeability, and so cause it to behave in an anomalous way.

2. When cells are suspended in NaCl, KCl, or glucose, changes in pH may occur, and these may be considerable. Particularly is this so in glucose, which is the very substance in which cation loss is most marked; the anomalous swelling of the cells in these media might therefore be related to undetected alterations in pH, bringing about changes in osmotic pressure.

3. Quite apart from these particular considerations, it is not only possible but probable that the red cell membrane, when surrounded by plasma, is a different membrane in a physical sense, from the membrane of the cell bathed in NaCl, KCl, or glucose. It would be very unsafe indeed to suppose that the properties of the membrane in these latter media would be any guide to its properties when in plasma.

Difficulties arise, however, when we try to measure red cell volume in hypotonic plasma. The colorimetric method cannot be used, haematocrite methods are worse than useless, and the diffraction method is not applicable to the discoidal cells in plasma. We have therefore to employ a different type of method in which we do not measure volumes in absolute units, but rather the percentage increase in volume, v, which results when the cell comes into equilibrium with the hypotonic plasma. These methods are three in number[17].

(a) If the cell takes in water from a hypotonic medium, its density will decrease, and from the extent of the decrease the amount of water taken in can be calculated.

(b) In a similar way the content of haemoglobin per unit volume of cells must diminish if the cells take in water, and from the extent of the diminution the amount of water which has entered can be computed.

(c) If the cell takes in water from a hypotonic solution, the amount of water taken in can be calculated from the figures for the water content of the cells in plasma and for the swollen cells in hypotonic plasma, both being obtained by drying a weighed quantity of cells to constant weight at 60°. Of the three methods, which all agree with each other sufficiently closely, the last is the most convenient.

When applied to erythrocytes in hypotonic plasma, all three methods give the same result, viz., the cells swell as if only 50 to 70 p.c. of their contained water were "free," a result very similar to that obtained in hypotonic NaCl and KCl, although perhaps a little nearer that which would be expected from a perfect osmometer. It is therefore clear that even in highly buffered plasma the cells do not follow Marriotte's law, but rather the law expressed in expressions (1, 2, 3 and 4).

I shall now summarise these results so far as the erythrocyte is concerned.

1. When the red cell is placed in an isotonic solution of NaCl, KCl, or glucose, it maintains its normal volume unchanged. The concentration of such an isotonic solution is one which is very nearly equivalent to the normal plasma of the animal from which the cells are obtained. It is difficult to be certain as to how nearly equivalent it is, and I have the impression that the isotonic saline solution is really a little more concentrated than the plasma; at all events, there is no great discrepancy.

2. When the cell is placed in a hypertonic solution of NaCl, KCl, or glucose, it shrinks less than it should if it obeyed simple osmotic laws, and its volume tends to increase with time. This is in accordance with the idea that cations pass through the membrane under such conditions.

3. When the cell is placed in hypertonic solutions of NaCl, KCl, or glucose, is swells much less than it ought to if simple osmotic laws are obeyed. We therefore suppose, with Kerr and others, that it loses cations or other osmotically active substances.

4. The cell nevertheless swells in a regular fashion as the tonicity decreases. This regular swelling could be accounted for by assuming that only part of the contained water is "free," or (since the idea of free water is not admissible), by supposing that the loss of osmotically active substances is a simple function of the tonicity.

5. This applies also to cells in highly buffered hypotonic plasma, although in this medium the extent of leakage appears to be slightly less.

The conclusion therefore is that the cell reaches equilibrium with a hypotonic environment in a way which cannot be accounted for by Marriotte's law, although it does so in a way which is susceptible of exact expression. I said a little time ago that it behaves in an orderly manner "according to a law of its own," but this, in a sense, is a misstatement, for several other types of cell behave similarly. Hill[12], for example, has shown that that same sort of thing is true for the muscle cell, which also swells less in hypotonic solutions than it ought to do if it followed simple osmotic laws. It behaves, like the red cell, as if only part

of its water were free, and Hill's explanation is essentially the same as that which I have given for the erythrocyte, viz., that there is a loss of cations. Siebeck[18] has reached the same conclusion, and given the same explanation, for the cells of kidney tissue, and Pantin[19] has used the same idea to account for the anomalous osmotic behaviour of *Gunda*. Even in the case of the cell which is supposed to be the typical perfect osmometer, the *Arbacia* egg, McCutcheon and Lucké[20] have found too small a degree of swelling if the egg is injured. If it makes the idea any more easy of acceptance, I am prepared to concede that a red cell, a muscle cell, or a kidney cell is "injured" when it is placed in a grossly hypotonic environment; again, however, I emphasize the essential point, that the effects of this "injury" are expressible quantitatively, and proceed in a regular fashion as the "injuriousness" of the environment changes.

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Discussion

Dr. Fricke: How long does it take to reach equilibrium in these hypotonic solutions? Could you follow, by any method, the volume changes which occur before equilibrium is reached?

Dr. Ponder: It takes about a minute to make a measurement of volume, diffractometrically, and by that time equilibrium has been reached in the systems which contain a large amount of NaCl and a small quantity of cells. I doubt if one could follow the volume changes; one would certainly need to devise a new method for doing so.

Dr. Cohen: Do you get intermediate values for cell volume in hypotonic solutions if you mix the NaCl and the glucose?

Dr. Ponder: Thus far I have not reached the stage of working with mixed solutions, but I imagine that one would get intermediate values of some kind.

Dr. Briggs: Is the NaCl solution, in which the cells do not either shrink or swell, exactly isosmotic with the plasma?

Dr. Ponder: That is a point upon which I would not like to commit myself, but it is certainly very nearly isosmotic with the plasma. The difficulty is that we can measure cell volume only to within about ± 2 p.c., and that the three volumes, 99 p.c., 100 p.c., and 101 p.c. would be indistinguishable from each other. These three volumes would nevertheless correspond to three different tonicities, and so it is impossible to say just what the tonicity for the maintenance of normal volume is. We can, however, usually pick out one NaCl solution, within this tonicity range, which has the same depression of freezing point as plasma.

Dr. Chen: Many preparations of NaCl contain silver, and it is known that this impurity has an effect on the haemolysis of fish cells.

Dr. Ponder: Kahlbaum's NaCl, I believe, is silver-free, and it was this preparation which I used.

Dr. Abramson: Is the leakage effect reversible?

Dr. Ponder: I do not know from my own experience, but it is stated not to be.

Dr. Abramson: In regard to the effect of the slide and coverslip on the red cells, are the cells, when they are spherical, very close to the glass surfaces?

Dr. Ponder: No. The diameter of the cell in its spherical form is about 5μ and the distance between the surfaces about 50μ. The cells move about freely between the surfaces.

Dr. Abramson: What I had in mind was that the potential on the glass surface might be responsible for the change in form.

Dr. Ponder: That has been suggested, but I do not think that it is so.

Dr. Abramson: Would the cells become spherical if you put the glass surface into plasma and had on it an adsorbed protein layer?

Dr. Ponder: The presence of plasma always prevents the assumption of the spherical form, but I do not think it is because it is adsorbed on the glass surfaces. It is rather an effect on the cell itself. Other proteins, such as haemoglobin and gelatin, are altogether without effect.

Dr. Chambers: One of the first papers on microdissection described the same sort of thing. If you approach a red cell with a microdissection needle, the cell undergoes crenation not unlike that which occurs before the spherical form is produced.

Dr. Abramson: Have you any explanation for the occurrence of the spherical forms?

Dr. Ponder: The only explanation I can offer is that it is a pressure effect. When the slide and coverglass are as close together as they are, the pressure between them may be considerable, and I fancy that this may in some way affect the cells lying between the surfaces.

Dr. Chambers: I have done rough measurements on *Arbacia* eggs in hypertonic, and hypotonic, NaCl solutions, and I have found that the shrinkage and swelling, in NaCl solutions, is less than it is in calcium chloride solutions. The changes in calcium chloride are reversible, but those in NaCl are not.

Dr. Ponder: McCutcheon and Lucké say that the *Arbacia* egg is a perfect osmometer in hypotonic sea water but I do not know that it follows that it is one in hypotonic NaCl. Under those circumstances the eggs may be injured, and this might account for the small swelling which you observe, and for its irreversibility. In fact, I am quite prepared to admit that when you place a cell in anything except plasma, or sea water, as the case may be, you are doing it an injury.

Dr. Cohen: Then one conclusion to derive from these results is that the integrity of the membrane, as an osmometer, depends on its external environment, as well as upon its structure.

Dr. Ponder: That is so, but it is not generally admitted, or, if admitted, is not sufficiently taken into account. The shape of the cell, and its metabolism, are influenced profoundly by the environment, and I have no doubt that almost every other property is affected as well.

THE EFFECT OF WING BUD EXTIRPATION AND TRANSPLANTATION IN CHICK EMBRYOS ON THE DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

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These experiments deal with the problem of determination of the nervous system. This organ is, in the vertebrate embryo, determined as a whole in the early stages of development. But that does not mean that all the qualitative and quantitative details of its pattern are already fixed at that time. We know that long after the formation of the spinal cord and the separation of the spinal ganglia these parts are—at least in their quantitative development—submitted to the influence of different factors.

We shall not deal with the group of *intrinsic* factors, working within the spinal cord itself. There exist growth correlations between the different levels of the spinal cord, which have especially been revealed by Detwiler (1) and other authors.

The same author and his collaborators have shown in extensive experimental work, that *extrinsic* factors, especially the peripheral fields to be innervated, also play an important rôle in the determination of the growth of the nervous system. To prove this, one has to decrease the peripheral field, for instance by extirpating a limb bud, or to increase it by implanting an additional one. The effects of such experiments are striking. Extirpation is followed by hypoplasia, additional implantation is followed by hyperplasia of the spinal ganglia concerned in their nerve supply. But this effect is, according to Detwiler's studies on *Amblystoma,* strictly limited to the spinal ganglia. The spinal cord does not react at all, either to decrease or increase of the peripheral field.

Whereas all students of the problem agree in the reaction of the spinal ganglia, Detwiler's statement concerning the spinal cord is *not* in agreement with other results. I shall only refer to the experiments of Miss Shorey (2), performed 25 years ago in Dr. F. R. Lillie's laboratory at the University of Chicago. She extirpated one wing bud in 72-hour chick embryos and found a remarkable hypoplasia, not only in the spinal ganglia, but also in the spinal cord, especially in the motor centers of the anterior horn of the operated side.

To settle these discrepancies, Dr. Lillie proposed to me to repeat these experiments. Miss Shorey had met with many difficulties in the technical procedure by using the thermocauter. The extirpation can be done much more easily and successfully by using a glass needle and a hair loop. The wing buds in 72-hour chick embryos can be removed by a single cut of the needle. The chorion heals well. Before the operation, a rectangular window is sawed in the shell, which is sealed in again after the operation. The embryos were fixed 5-6 days after operation. They show in most cases complete absence of the wing. One can increase the loss of muscles by additional extirpation of the anlagen of the shoulder girdle and its muscles. This variation proved to be valuable for further analysis.

Histological study of four cases revealed the following facts: On the operated side, a variable portion of the shoulder girdle and its muscles is present. The spinal nerves of the wing level are much smaller on the operated side, but a normal brachial plexus is formed. The spinal ganglia, Nos. 13-16, show a remarkable hypoplasia. The spinal cord is strikingly affected too. Four groups of cells may be distinguished in it:

(1) The most obvious hypoplasia can be seen in the *lateral motor group,* supplying the wing. The number of neurones is considerably reduced.

(2) The *median motor group* is not affected. These neurones supply the axial trunk muscles whose anlagen were not injured in the operation.

(3) The size of the *posterior horn* is reduced.

(4) The *medial part* of the cord is not affected.

These data completely confirm Miss Shorey's results in all details. They also agree with the results of R. May (3), who recently reported motor cell hypoplasia in frogs following limb bud extirpation. The contradictory results of Detwiler concerning *Amblystoma* cannot be explained at this time.

It seemed to be interesting to follow the hypoplasia more in detail, especially that of the motor region, and to compare it with the actual muscle loss. To get exact quantitative data, counting of cell nuclei was begun in the spinal ganglia and the cord, and the muscle loss was calculated by drawing the muscles on cardboard, cutting them out and comparing the weight of the right and left brachial muscles.

The hypoplasia in the lateral motor group, ranging from 62% to 30%, corresponds quantitatively to the loss of muscles, ranging from 92% to 43%; whereas the hypoplasia in the spinal ganglia does not vary, showing an average of 35%. This is to be expected, as the skin loss is nearly the same in all cases, independent of whether or not the shoulder muscles are present.

These quantitative studies seem to indicate a correlation between each part of the peripheral field and its own nerve center. The muscles ap-

parently control the growth of the motor centers, and the skin controls that of the sensory center, and both actions may be independent of each other. I hope by continuing these quantitative studies to approach the main problem of this field, the problem of the mechanism by means of which the peripheral field manages to stimulate the growth of the nervous system. The argument would be as follows: If the different parts of the peripheral field influence their own nerve centers directly, then no other path of transmission for these stimuli need be imagined than the nerves themselves connecting the peripheral areas with their centers.

Transplantations. It was to be expected that, as in amphibians, the overloading of the periphery in a given region, for instance by the implantation of an additional limb, might result in hyperplasia of the corresponding part of the nervous system. Therefore a series of hind limb and wing transplantations was performed. A limb bud was cut out of a 72-hour chick embryo and transplanted to another embryo of the same stage. Laterally, between wing and hind limb bud, there is just room for a third bud (For details of technique see (4)). The transplant is allowed to develop for 4-6 days and often grows normally.

Only two cases have been sectioned up to the present time. Both show normal histological differentiation of muscles, cartilage, etc. One of them is completely nerveless, thus proving, as has been shown for frog development (5), that normal formation and histological differentiation are independent of nerve supply. The other transplant is innervated by two small nerves, emerging from the spinal cord at the nineteenth and twentieth segment. The two spinal ganglia, numbers 19 and 20, show an increase in cell number of 28% and 23%. The spinal cord has not yet been studied.

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(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 8).

THE DIFFERENTIATION OF THE PROTOPLASM OF EGG CELLS DURING EARLY DEVELOPMENT

(*Continued from Page* 265)

the formation of the polar bodies. After the phenomena of maturation, some of the egg substances begin to migrate towards the pole where the polar bodies have been formed, the others towards the opposite pole. This migration holds for both microscopical and ultramicroscopical particles. A series of experiments showed that this new arrangement cannot be caused by gravity, nor by differences of the surface tension. Its formation also is too complicated to be explained by diffusion phenomena. This conclusion alone makes it probable that the cause of this so-called "bipolar differentiation" must be some electrical phenomenon.

In a study of the bipolar differentiation of the eggs of the neapolitan *Nereis Dumerilii,* it could be shown that the cell substances of these eggs were being distributed in such a way that substances of a high pH migrated towards the animal pole, substances of a low pH towards the other pole. In the egg cells of *Nereis Dumerilii* there is a homogeneously dissolved yellow pigment which is a natural indicator changing color at about pH 5.4 from yellow to violet from the alkaline towards the acid side. During the bipolar differentiation the pigment is accumulated in the vegetative half of the egg cell, while the animal half becomes colorless. The first four blastomeres separated by meridional cleavage furrows show essentially the same organization. The bipolar differentiation of these blastomeres is still proceeding. When the accumulation of the substances on the vegetative pole reaches a certain amount, the color of the natural pigment changes from yellow to violet, indicating an increase of acidity, i.e., a pH of at least 5.4 in the vegetative part of each of the four blastomeres. This happens just before the third cleavage. Vital staining experiments with indicators also show an *increased acid reaction of this part of the blastomeres* from this time on. Moreover, these dyes also stain the other (animal) half of the blastomeres and here they indicate an *alkaline color* more pronounced than before. Each of the four blastomeres now consists of an alkaline part and an acid part. Between them the indicators have an intermediate color.

The preliminary explanation of these observations is that the bipolar differentiation of the egg cells is a *cataphoretic phenomenon,* that somehow there is established in the living cell an electrical field and that this causes a migration of the mic-

roscopical and ultramicroscopical particles according to their electrical charge. The particles of cell substances which show an alkaline reaction migrate in one direction, those of the acid substances in the other. The different cell substances apparently can have a very different pH, even if they are enclosed in one single cell. The establishment of the electrical field is somehow tied up with the presence or local concentration of certain ions, since it is possible to start the bipolar differentiation of egg cells of many different animals artificially by adding KCl to the culture medium.

The succeeding cleavage furrows separate the two different parts of the *Nereis* egg. In this way there originate two different groups of blastomeres, one, after vital staining with indicators, showing the color of the acid side and the other showing the color of the alkaline side. During the later development the alkaline cells form the ectoderm, the acid cells the entoderm. It seems to be important that during segmentation bipolar differentiation phenomena still proceed in the single blastomeres in the same sense as in the undivided egg cell.

These observations were first done on eggs of *Nereis Dumerilii*. In that first study only two vital staining indicators were used, namely, neutral red and Nile-blue sulfate. In later investigations brilliant vital red, brilliant cresyl violet and cresylecht violet were also used as vital staining indicators with very good results. Brilliant cresyl violet and cresylecht violet have peculiar properties which one must know if one wants to use these dyes as indicators (see *Protoplasma*, 1933, **18**, 497). In some cases it is possible to obtain a beautiful vital stain with methyl red and brom cresol purple by adding the dyes to pure isotonic NaCl solution instead of to the normal culture medium. All the experiments described with reference to the eggs of *Nereis Dumerilii* have been repeated with the whole series of indicators mentioned above (with the exception of brom cresol purple) on the egg cells of *Nereis limbata* in Woods Hole with essentially the same results. Similar studies on the eggs of *Chaetopterus pergamentaceus* showed that the principle of the bipolar differentiation of these eggs is the same as in the *Nereis* eggs.

Eggs with a discoidal type of cleavage, as for example, those of the *Teleosts* and *Cephalopods* are exceptionally good material for studies on the bipolar differentiation. A great number of experiments were done on eggs of fresh water fishes in Munich and Heidelberg, and a series of vital staining experiments on the eggs of *Loligo vulgaris* at Woods Hole. The results obtained on fish eggs are important for the analysis of the phenomena, because on this material it is possible to control the vital staining with indicators by microinjection of the whole series of the pH indicators of Clark and Lubs, and to vary and analyze the method from different physical viewpoints. Hydrogen ion determinations with both the vital staining indicators and also with the Clark indicators gave the same results. In both the fish eggs and the *Loligo* eggs, alkaline colloidal substances migrate after the formation of the polar bodies towards the animal pole, and acid substances towards the vegetative pole. The protoplasm of the animal cap of the trout eggs has a pH of more than 7.6; the yolk substances have a pH of about 5.6 or less.

In the egg cells which have a concentric organization, the substances of the cortical layer and those of the central part of the cell body show also a great difference in pH, and are distributed in the egg cell according to their pH. Either the alkaline substances are accumulated below the surface and the acid colloids in the center of the cell body, or—in the eggs of other animals—the cortical layer shows the more acid reaction and the central part of the cell the alkaline one.

In some types of cells of *Teleosts* (unripe ovary eggs) it was possible to show that even if the protoplasm is hyaline and microscopically not differentiated at all, it contains several substances or phases which have a very different pH. (Colloid particles of different reaction must somehow be protected from each other in the living cell.) This fact seems to be of great importance for the whole analysis of differentiation phenomena and obliges us to review our methods and interpretations of pH determinations in living cells.

(This article is based upon a lecture presented at the Marine Biological Laboratory on August 11).

GENERAL SCIENTIFIC MEETING

THURSDAY, AUGUST 31, 1933

Part I. 9:00 A. M.

1. Dr. Paul Reznikoff and Mrs. Dorothy G. Reznikoff: Blood cell studies in dogfish.
2. Dr. W. H. F. Addison: Intracranial pigmentation in teleosts.
3. Dr. E. R. Clark and Mrs. Eleanor Linton Clark: The blood capillary in relation to contractility.
4. Mr. Herbert L. Eastlick: Striated muscles of the lamellibranch mollusc, Pecten gibbus.
5. Dr. Arthur W. Pollister: The centrioles of amphibian tissues.
6. Mr. Theodore G. Adams: The chromidium in Arcella vulgaris.

(Continued on Page 302)

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Jean M. Clark, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

Printed by the Darwin Press, New Bedford

A NOTE OF APPRECIATION

The lecture and motion picture on whaling lore was successful. The auditorium was comfortably full, and the value of the tickets sold amounted to $207.00. The expenses of the show, counting the fee for the lecturer, publicity and miscellaneous expenses, was approximately $70.00. This enables us to deposit more than $135.00 to the account of THE COLLECTING NET Scholarship Fund in the Falmouth National Bank, and makes certain again of the award of six one-hundred dollar scholarships for the students of the Marine Biological Laboratory and of one for the Biological Laboratory at Cold Spring Harbor.

We wish to express our deep appreciation to Dr. Conklin for his brief introductory talk and to thank sincerely all those who contributed to the success of the performance: especially Mr. Howland, the lecturer; Mr. Sherman, who brought down the exhibit of whaling equipment; Miss Emily Ann Lillie and Miss Ruth Burdett for their posters; and to Margaret and Kathleen Stokey, who sold many tickets for us.

At this time we would like to acknowledge two gifts, each of ten dollars, to the Scholarship Fund from two Falmouth merchants, Dr. E. C. Cole and the Wood Lumber Company.

Introducing

DR. REID HUNT, professor of pharmacology at the Harvard Medical School, who is spending a few days here at Woods Hole, renewing old acquaintanceships and reading in the library of the Marine Biological Laboratory. Dr. Hunt is especially interested in drugs which act upon the parasympathetic nervous system, particularly in derivatives of choline. He was the first to discover the extraordinary activity of acetyl choline, which is perhaps the most active drug made. It is now being used to some extent in medicine. Dr. Hunt first discovered this compound many years ago in the adrenal glands; it is now classed with the hormones and is believed to be an important factor in connection with the circulation. He has also done much important research work on the thyroid gland and is in addition noted for his work on nitrites, quinine, arsphenamin, alcohol, and the biological standardization of drugs.

Dr. Hunt arrived in Woods Hole on August 17th. He plans to leave early in September.

GENERAL SCIENTIFIC MEETING

(Continued from Page 301)

7. Dr. Harold H. Plough. Selective fertilization in Styela.
8. Mr. M. Atlas: Relation of temperature and cleavage in frog's eggs.
9. Dr. E. G. Conklin: Disorientations of development in Crepidula, caused by cold.
10. Dr. Ethel Browne Harvey: Changes in the Arbacia egg immediately following fertilization, as determined by centrifugal force.
11. Dr. P. S. Henshaw and Dr. D. S. Francis: Recovery from X-ray effects before fertilization in Arbacia eggs and its effect on development.
12. Miss Anna K. Keltch, Miss Lucille Wade and Dr. G. H. A. Clowes: Further observations on the contrasting sensitivity of eggs and sperm to various chemical agents.
13. Dr. G. H. A. Clowes, Miss Anna K. Keltch and Miss Lucille Wade: Variations in the sensitivity of eggs following fertilization.
14. Dr. E. Newton Harvey: Flattening of marine eggs under the influence of gravity.
15. Dr. L. V. Heilbrunn: The action of anaesthetics on the surface precipitation reaction.
16. Dr. Dorothy R. Stewart and Dr. M. H. Jacobs: The effect of certain salt solutions on the permeability of the Arbacia egg.
17. Dr. B. R. Speicher: The effective period in development of the mutant factor "eyeless" in Habrobracon.
18. Dr. Anna R. Whiting: Variegated eye color in Habrobracon.
19. Dr. P. W. Whiting: Egg-trinuclearity in Habrobracon.

Intermission.

Part II. 2:00 P. M.

20. Mr. Heinz Specht: Relation between oxygen tension and respiration in Spirostomum ambiguum, with corrections for Ammonia.
21. Dr. C. S. Shoup: Respiration and luminescence of bacteria in carbon monoxide.
22. Dr. G. Wellford Taylor: The relation between luminescence and respiration in bacteria with especial reference to the effects of narcotics.
23. Dr. Lyle V. Beck: Nature of the aerobic apparent reduction potential.
24. Dr. Eric G. Ball: The relative abundance of hydrogen isotopes in sea water.
25. Dr. Oscar W. Richards: Toxicity of some metals and Berkefeld filtered sea water to Mytilus edulis.
26. Dr. Herbert H. Jasper: Some new aspects of the physiology of the nerve-muscle system in crustacea brought out by electrical excitation and response.
27. Dr. Margaret Sumwalt and Miss Kathryn McLane: The blood pressure of Limulus.
28. Mr. John C. Bridges and Dr. Margaret Sumwalt: The effect of pH upon potassium penetration into Fundulus eggs.
29. Dr. Arthur K. Parpart: A method for following volume changes of cells.
30. Dr. William R. Amberson, Mr. Frank Engel, Miss Dorothy Webster, and Dr. Edwin P. Laug: The influence of pH upon the passage of hemoglobin through the glomerulus of the perfused frog's kidney.
31. Dr. M. H. Jacobs and Dr. Arthur K. Parpart: The influence of the escape of salts on the osmotic behavior of the erythrocyte.

THE COLLECTING NET

Vol. VIII. No. 10 SATURDAY, SEPTEMBER 2, 1933 Annual Subscription, $2.00
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THE COPEPOD PLANKTON OF THE LAST CRUISE OF THE NON-MAGNETIC SHIP "CARNEGIE"

Dr. C. B. Wilson
State Teachers College, Westfield, Mass.

The last cruise of the non-magnetic ship *Carnegie* covered portions of the Atlantic Ocean north of the equator, and of the Pacific Ocean between latitudes 52° North and 40° South.

During the entire cruise plankton was collected at the surface and at depths of 50 and 100 meters and at the same time data were obtained of the temperature, salinity, density, hydrogen ion, and phosphates at the three depths.

As the towings were all made in the daytime by the same persons, with the same nets, using the same methods, and in as close succession as possible, they furnish the best basis thus far obtained for a comparison of the plankton of the two oceans, and of the different portions of each. An examination of the copepods of this plankton, just completed, yields the following general results.

1. The Pacific plankton is 50 – 100% richer than that of the Atlantic, both in number of species and in number of individuals. On the other hand, there was not found in the Pacific any trace of such countless swarms of a single species as are often seen of *Calanus finmarchicus* at certain seasons in the northern Atlantic.

2. The plankton of the southern Pacific is richer than that of the northern portion, and of course the tropical (*Continued on Page* 347)

THE HORMONS OF THE PITUITARY AND THE THYROID[1]

Marie Krogh, M. D.
Lecturer in Physiology; State School for Teachers; Examiner, University of Copenhagen

Our knowledge about a thyroid stimulating substance from the hypophysis is of a fairly recent date. The first report concerning an effect of extracts of the anterior pituitary has been given by Leo Loeb and his associates, and shortly after, independent of the previous paper, appeared a paper by Aron on the histological changes in the thyroid caused by injection of anterior pituitary extract.

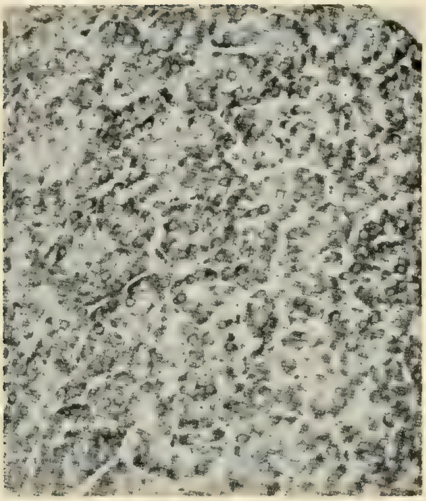
At this time, Dr. Harald Okkels and I had been working together on the thyroid problem, examining surgically removed human goiters, Dr. Okkels from a cyto-histological point of view and I studying the metabolic effect of the glands fed to guinea pigs in equi-iodine amounts.

The results obtained were that in human exophthalmic goiters, contrary to colloid goiters, the Golgi apparatus in the thyroid cells was distinctly hypertrophic, indicating a hyperactivity of the cells, and, furthermore, that the metabolic effect of the dried gland fed in equi-iodine doses was less for the colloid-poor or nearly colloid-free exophthalmic goiters than for the normal thyroid and for the colloid goiters.

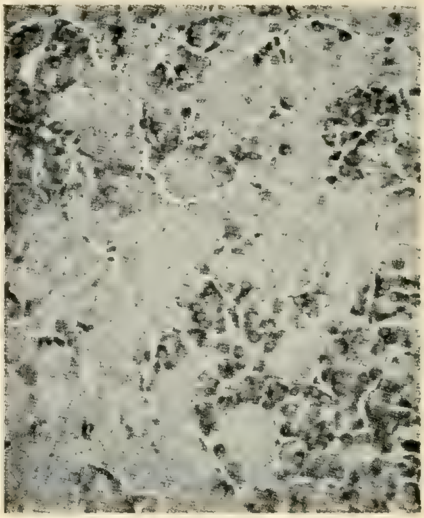
So far, our results combined with earlier known histological changes in the thyroids and

[1] These experiments have been made in collaboration with Dr. Harald Okkels.

TABLE OF CONTENTS



Normal guinea pig anterior pituitary 200x.



Anterior pituitary of guinea pig treated with 1000R 200x.

Figure 3

with the clinical symptoms emphasized the difference between the exophthalmic goiter and all other types of goiters.

We therefore welcomed the possibility of producing thyroid hyperactivity by means of anterior pituitary extracts, and carried out a series of experiments according to the following scheme: (1) examining the influence on the metabolism; (2) the influence on the Golgi apparatus in the thyroid cells; (3) the influence on the other morphological conditions in the thyroid.

As experimental animals in the experiments I am going to report this evening we used full grown guinea pigs which we have found to be suitable animals because of the constancy of their metabolic rate. The recording respiration apparatus is connected with two or three respiration chambers, which can be used successively.

After injection of anterior pituitary extracts we found: (1) Increase of the standard metabolism; (2) Enlargement of the Golgi apparatus in the thyroid cells; (3) Increased vascularisation, cell hypertrophy and diminution of the colloid content.

In order to obtain an absolutely objective estimation of the histo-cytological conditions, my assistant, Miss Lindberg, and I remove the thyroids from the experimental and control animals and send them to Dr. Okkels, who is examining them under a number without knowing what has been done with the animals until he has made a written report on the microscopical findings.

TABLE I

ADULT MALE GUINEA PIGS

	Number of treatments	Amount	Average increase over standard metabolism ccO_2 per sq. m/min.	Cytological changes	Histological changes
injected ant. pit.	7	4.3 g	+39	+++	++++
fed ant. pit.	7	4.3	+1	0	0
injected prolan	6	180 m.u.	0	0	0
fed dried thyroid	7	0.78 g	+48	0	0

Table 1 shows the typical effect of injection of anterior pituitary, on the metabolism, on the Golgi apparatus, and on the other morphological conditions, colloid resportion, et cetera. Anterior pituitary extract by mouth has no effect; 180 mouse units of Prolan injected have no effect either. Feeding dried thyroid causes, as is known, a considerable metabolic rise, but no hyperactivity of the cells; on the contrary, we found that after feeding dried thyroid for eight months the guinea pig's thyroid showed atrophic changes.

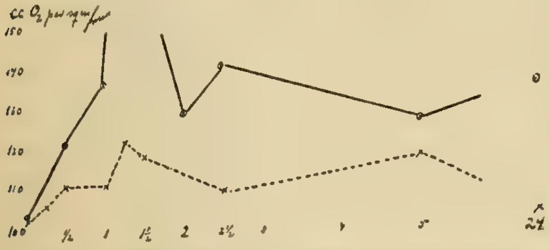


Fig. 1. Metabolism of adult male guinea pigs measured during the first 5 hours and 24 hours after intraperitoneal injection.

1. ⊙ injection of ant. pit. extract (average of 4 animals).

2. x injection of Ringer solution (average of 3 animals).

The effect of the thyro-stimulating hormone becomes visible very soon after injection; as early as twenty to thirty minutes after intraperitoneal injection the cells have changed, and after one hour the Golgi apparatus is enlarged and the colloid resorption pronounced. A considerable metabolic rise compared with that of control animals injected with Ringer's solution indicates that an increased amount of thyroxin is given off into the blood stream.

Having convinced ourselves about the fact that injection of anterior pituitary induces thyroid hyperactivity, we carried out some experiments in order to contribute to the exophthalmic problem.

In preliminary chemical experiments we found that the thyroid stimulating substance in the presence of protein can be precipitated by 70% alcohol but dissolved in 48% alcohol. Combining the alcohol method with the trichloracetic acid and acetone method, indicated by Loeser, we were able to get a protein free preparation of which about 4 mg. is enough to produce thyroid hyperactivity in guinea pigs.

In contrast to Aron we had been unable to detect the thyroid active substance in fresh untreated urine. We therefore made use of the information gained about the chemical properties and tried to concentrate the substance in urine from patients with severe attacks of exophthalmic goiter and from guinea pigs injected with large doses of anterior pituitary extracts.

As shown in Table 3, the urine concentrates did not cause changes at all in the thyroid gland. The explanation I can offer for the slight metabolic rise is that the fairly large amounts (about 15 cc. per day) of the unsterilized residue after the alcohol evaporation produced some tissue irritation, and the animals therefore did not keep as quiet during the metabolism determinations as required for standard conditions. The control experiment with urine plus anterior pituitary extract shows that the urine itself does not inactivate or destroy the thyroid stimulating substance.

We therefore find it legitimate to conclude that the thyroid stimulating hormone is not eliminated by the kidneys.

Dr. Okkels previously found on surgically removed human thyroids that after the Plummer

TABLE 2

Adult male guinea pigs daily treated with purified anterior pituitary preparations

Evans alkaline extract, treated with:	Number of injections	Total amount mg	Average increase over standard ccO$_2$ per sq. m/min.	Cytological changes	Histological changes
70% alcohol, trichloracetic acid, acetone	3	4.2	+23	+++	++
48% alcohol, 70% alcohol, trichloracetic acid, acetone	3	6.6	+26	++++	++++

TABLE 3

Urine from patients with exophthalmic goiter and from guinea pigs with experimental hyperthyroidism. (Urine +1% plasma, precipitated with 70% alcohol and extracted with 48% alcohol.)

	Total amount of urine cc.	Number of injections	Average increase over standard metabolism ccO$_2$ per sq. m/min.	Cytological changes	Histological changes
Urine from Patient 1	2830	4	+3	0	0
" 2	3170	4	+7	0	0
" 3	5150	4	+19	0	0
Urine from 4 guinea pigs injected 4x23cc. ant. pit. extract	510	4	+19	0	0
4 guinea pigs injected 4x16 ant. pit. extract	725	5	+13	0	0
Normal urine +3x8cc. ant. pit. extract	2365	3	+41	++++	+++

pre-operative iodine treatment of exophthalmic goiter, there is no diminishing of the enlarged Golgi apparatus in spite of colloid storage in the alveoles. This means that the cells are still hyperactive, but to a certain extent able to regulate the amount of thyroxin given off into the blood stream. With this in mind, we tried to imitate the Plummer treatment on guinea pigs with experimental hyperthyroidism. From our previous experiments we knew that for full grown guinea pigs injected daily with 2-3 cc. of the Evans alkaline extract, the metabolism rises to an average of about +40%, and the thyroid is morphologically changed often to such a degree that it cannot be distinguished from the picture of a human exophthalmic goiter. We therefore injected daily anterior pituitary extract into the animals and after 4-5 days, when the metabolism was about +40, we started iodine treatment, continuing the anterior pituitary injections.

Figure 2 shows that the metabolism of a guinea pig with experimental hyperthyroidism reacts to iodine like that of an exophthalmic goiter patient. But when the thyroitoxic condition is produced by feeding thyroid preparation, the iodine does not lower the metabolism—an effect similar to that in human toxic adenoma. The microscopical examinations of the iodine treated experimental hyperthyroidism show the same picture as the iodine treated exophthalmic goiter, namely, an accumulation of colloid but persistence of the enlargement of the Golgi apparatus. I shall add that in addition to a number of experiments like the one shown on the figure, where the animals were killed as soon as the metabolism went down to normal, we carried out just before I left Copenhagen an experiment lasting for 44 days, where the guinea pig was injected with anterior pituitary extract and fed iodine. It kept a normal metabolism and a good health, while a control animal injected for a fortnight with the same daily amount of anterior pituitary, but without having iodine, had an average metabolism of +42% and loss of weight and hair.

We are, of course, going to repeat this experiment to make sure whether or not a suitable iodine dose is able to control or neutralize the thyroid stimulating hormone for a longer time.

In collaboration with an X-ray expert, Dr. Arntzen, we have tried to eliminate the anterior pituitary effect on the thyroid by exposing the hypophysis to an elective X-ray treatment.

The smaller doses have no or uncertain effect, but doses of about 1000R cause damage to the hypophysis and corresponding to this effect we find a metabolic drop of 13-14% below normal, atrophic changes of the thyroid cells and of the Golgi apparatus. To confirm the results we are going to repeat these experiments and also for the reason that X-ray treatment may be useful in inoperable cases of exophthalmic goiter.

In our opinion the results make it probable that exophthalmic goiter as distinct from other types of goiter has its origin in anomalies in the function of the anterior pituitary gland.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 15).

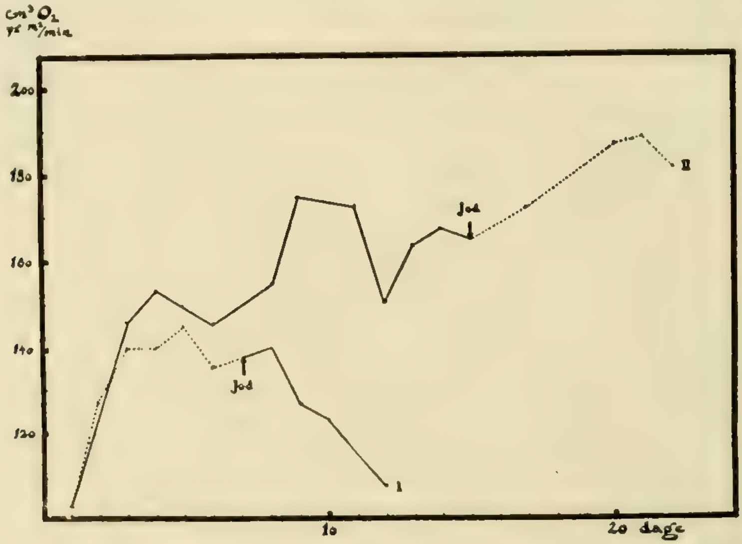


Fig. 2. Standard metabolism of guinea pigs, I., injected ant. pit. and fed iodine, II., fed dried thyroid and iodine.

THE BIOLOGICAL LABORATORY

COLD SPRING HARBOR

REVERSIBLE TWO-STEP OXIDATION

LEONOR MICHAELIS

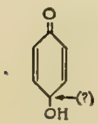
In general, an organic dye stuff of quinoid structure is reduced by accepting from the reducing agent two hydrogen atoms or two electrons at once. Until recently no case had been known in which the reduction would occur in two separate steps in succession, each involving the acceptance of one electron (or hydrogen atom). This experience seemed to be quite natural and in agreement with the customary formulae, used in organic chemistry, for a quinoid or a benzoid compound. No intermediary form seemed to be imaginable, because one valence in this imaginary "semiquinoid" form would have to stand aloof and unsaturated:

Benzenoid structure

(hydroquinone)

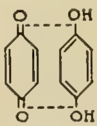
Quinoid structure

(quinone)



Hypothetical semiquinoid structure with one unsaturated bond.

However, a substance at an oxidation level intermediary between the benzoid and the quinoid form had been known for a long time, to wit, quinhydrone. In order to avoid the seemingly unacceptable semiquinoid formula Willstaetter and Piccard proposed the following structure for

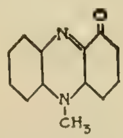


Quinhydrone as a "meriquinone"

quinhydrone. One molecule of quinone and one of hydroquinone were supposed to be combined to form one molecule of the double size, held together by some kind of residual valences. The term "meriquinone" implied that the oxidized half and the reduced half of this double-molecule may not be fixed but shifting continuously so as to distribute the state of oxidation in the time average equally between both halves of the molecule. There was, however, no definite proof for this hypothesis because of the fact that quinhydrone exists only in the solid crystalline state in which no determination of molecular weight can be performed. In the dissolved state it is completely "dissociated" into hydroquinone and quinone.

In the meantime other substances at the semiquinoid level of oxidation have been discovered, which are much more favorable for the study of their structures. Some of them are constituents or products of living organisms. The number of cases of this kind is increasing at such a rate that there can be no doubt of a much wider occurrence in living organisms than has been known as yet. There can be little doubt, furthermore, that the occurrence of such substances in living cells has some biological significance. I am thinking of quite a definite significance, of quite a definite rôle played by this kind of substance in the process of oxidation metabolism. This, however, is still a vague idea and has to wait for further elaboration. Therefore, I shall, at the present time, refrain from discussing the biological importance and present only the chemical side of the problem with the definite idea in mind that this matter will very soon be taken up successfully from the biological standpoint.

The particular substance in which the first observation pertaining to this problem has been made is the blue pigment pyocyanine produced by *Bacillus pyocyaneus*. In a systematic search for reversible oxidation-reduction systems it was decided to study this pigment, which was known to be easily reducible to a leuco-compound and easily re-oxidizable. The chemical constitution of this dye has been found by Wrede and Strack to be N-methyl α-oxyphenazine. These authors indeed attributed to it the double molecular size on the ground of an apparently inadequate determination of its molecular weight. The method about to be presented shows that the simple structure of the following formula is correct:



This is an orthoquinoid molecule and could be expected to behave like a regular quinoid dye stuff

when subjected to a reductive or oxidative potentiometric titration such as inaugurated by W. Mansfield Clark and his associates, especially Barnett Cohen. As a matter of fact, this was true provided the titration was executed in an alkaline solution. In acid solution, an unexpected phenomenon occurred, which seemed to be quite unique at that time but has in the meantime turned out to be one instance among many others. The color of pyocyanine at pH < 4.9 is red. When it is being reduced, it first turns green and then colorless. In strongly acid solutions these two steps are distinctly separate, in less acid solutions they overlap and with increasing pH the overlapping becomes so complete that the green intermediary form does not appear at all during the course of the titration.

It was very tempting to ascribe to this green intermediate form the meriquinoid structure of Willstaetter and Piccard. This suggestion could easily be tested by an analysis of the titration curve, the potentials being plotted against the percentage of oxidation or reduction. Let us first consider which curve can be expected on the basis of such an assumption. The reversible chemical reaction in the oxidation of the completely reduced form to the intermediary form would be:

2 Mol Reduced form $\rightleftharpoons$ 1 Mol Meriquinone + 1 H

Hence, the potential during the first step of oxidation must depend on the ratio of the two forms, when pH is kept constant, in the following way:

$$E = \text{Constant} + \frac{\mathbf{R}T}{\mathbf{F}} \, ln \, \frac{(\text{Meriqn})}{(\text{Reduced form})^2}$$

This formula shows that the potential depends not only on the ratio of the two forms, as is usual in other oxidation-reduction systems, but also on the absolute amounts. In the second place, it shows that the potential curve is not symmetrically arranged around the mid-point of the titration. The experiment, however, was not in agreement with this formula, as shown in the following graph (Fig. 1). This curve shows the two steps of oxidation as they occurred in a very acid solution. The curve of the first step should behave as has just been demonstrated, but it does not. The second step should behave also as developed before, but it does not either. Each of the two steps shows a course perfectly symmetrical around the individual mid-point, and the whole curve is the same no matter what the total concentration of the dye at the start of the experiment. The slope of each half of the curve is the one for an oxidation involving only one hydrogen atom or electron, and of necessity the above chemical equation must be discarded and replaced by the following much simpler one:

1st step: 1 Mol reduced form $\rightleftharpoons$ 1 Mol intermediate form + 1H;

2nd step: 1 Mol intermediate form $\rightleftharpoons$ 1 Mol oxidized form + 1H.

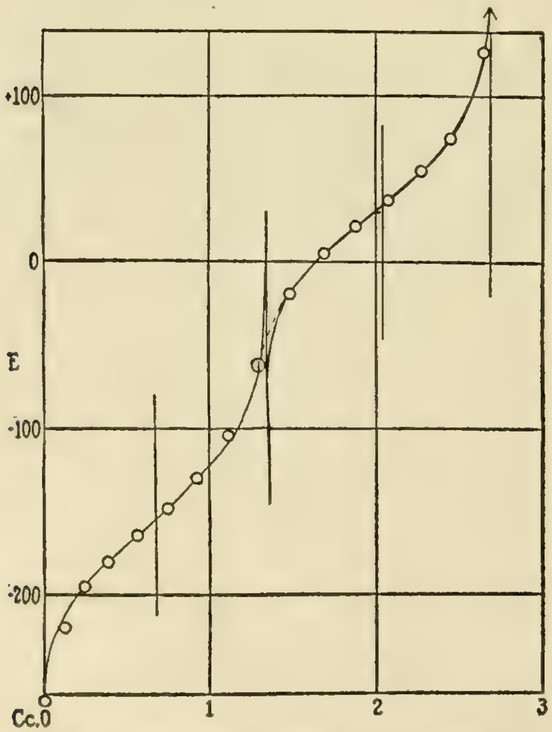
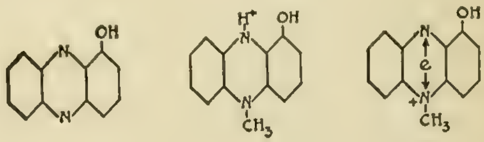


Fig. 1. Electrometric titration curve obtained when reduced pyocyanine is titrated with $K_3Fe(CN)_6$ at pH 1.82.

This shows that the intermediate form has the same molecular size as either the reduced or the oxidized form, and that the intermediate form differs from either the reduced or the oxidized by nothing but one H-atom (or one electron). The intermediate form therefore must have the seemingly unacceptable formula with a semiquinoid structure and is therefore of the character of a chemical radical, which may be written as follows:



I II III

Formula I shows the simplest possible way of writing. It contains what we may call a bivalent

nitrogen atom. On considering that this semiquinoid form appears only in acid solution, we may add a proton to it in the same way we add a proton to ammonia to make it an ammonium ion (see Formula II). In order to explain the unexpected stability of such a radical we may add the following hypothesis: the positive charge is not definitely located at the one nitrogen atom but shifts periodically to and fro between the two nitrogen atoms, as a result of a periodic oscillation of one electron between two N-atoms. Each nitrogen atom may be said to possess an outer electron shell of only seven electrons, which by the odd electron is alternately supplemented to a regular octet. This odd electron, loosely held and oscillating with a rather slow frequency may be imagined to be responsible for the very intense color of all these semiquinoid compounds and for the very distinct band spectrum exhibited by most, though not all, of the semiquinones. Radicals of this kind are not entirely unknown in organic chemistry. The best known instances are triphenyl-methyl, discovered by Gomberg, and diphenyl-nitride, discovered by Wieland. These radicals are as a rule in part associated as double molecules of a saturated character. Such an association, however, does not take place in the semiquinoid radicals, obviously for electrostatic reasons. These radicals are monovalent cations and it is unlikely that two monovalent cations would combine to form a bivalent cation.

Once this phenomenon had been observed in the particular case of pyocyanine, many other examples were found. In the first place, a great number of phenazine compounds turned out to behave in the same manner, among them also the simple phenazine itself. Practically all those phenazine compounds show this phenomenon, except those containing one amino group or several as a side chain. To this group there belongs also, according to Kögl, another bacterial pigment, chlororaphine. Of other groups of organic compounds which can be oxidized through the oxidation level of a semiquinone are the aromatic paradiamines. A certain difficulty is involved in the study of these compounds because of the fact that the fully oxidized forms, at the oxidation level of a quinone, are very labile substances undergoing a rapid irreversible disintegration. The semiquinoid forms are what were called a long time ago Wurster's dyes, which were considered by Willstaetter and Piccard as double molecular meriquinones as a result of a now well understandable misinterpretation. When for each of the two H-atoms in the two amino groups, phenyl groups are substituted, all of the three possible forms are perfectly stable molecules, the reduced, the semiquinoid and the quinoid forms.

Another especially interesting group of this kind are the derivatives of γ-γ'-dipyridyl, especially the quaternary ammonium derived from it. In these cases, in contrast with all the others, it is the reduced form which exhibits the quinoid structure, and it is the oxidized form which has the benzenoid structure. The form intermediary between these two, the semiquinoid, is an intense blue-violet dye stuff, whereas both the fully oxidized and the fully reduced forms are colorless. The fully reduced form happens to be a rather labile compound, whereas the two other forms are perfectly stable substances. The preparation practically used is the oxidized form; on reduction it turns blue-violet reversibly. The normal potential of this first step of reduction is extremely negative, amounting to about −.45 Volt, independent of pH within any practically available range. This potential is, at pH 7, virtually equal to the potential of a hydrogen electrode at one atmosphere pressure. It is easy to see that under these circumstances the normal potentials of these substances are in the range of hydrogen overvoltage in acid solution but, on the other hand, are more positive than the hydrogen potential in an alkaline solution. The very negative range of potential makes these substances very desirable oxidation-reduction indicators for potential range not accessible to the well known indicator series established by W. M. Clark and associates. For brevity's sake they may be called viologens. I have prepared methyl-, ethyl-, benzyl-, and betain-viologens, of which the benzyl compound has a normal potential about ninety millivolts more positive than the three others, all of which are close to −.45 Volt. The substances are relatively very little poisonous and I hope they may become useful as indicators in biology.

A mathematical analysis of the oxidation or reduction titration curve has been developed for those cases in which all of the three forms are stable and we have to deal with true equilibria. The result of such an analysis is very striking and most instructive for various problems. It will especially find application in what is called the Cannizzaro reactions, in which two molecules of the same kind act upon each other in such a way that one is reduced and the other is oxidized, so that the original substance is on an oxidation level intermediary between the two others. The mathematical theory cannot be given in a lecture with any profit to the audience, and may be studied in the original papers by myself and by Elema; but I wish to demonstrate some of the results.

Suppose we have a substance in its reduced form and we titrate it with an oxidant. Let this substance be capable of forming two successive steps of oxidation, each one by acceptance of one

electron. There are two limiting cases possible. In one case, on oxidation the semiquinone alone is formed first and after the whole of the substance has been oxidized to the semiquinone the oxidation to the higher level begins. In this case the curve of the potential plotted against percent of oxidation distinctly shows two separate steps, each of which has the character of an ordinary titration curve in a one electron system, such as ferrocyanide-ferricyanide. In the other limiting case, upon addition of the oxidant, the second step of oxidation is formed already before the first step is completed. In this case, an overlapping of the two steps takes place, and this overlapping may reach different degrees of completion, until it is so strong that the intermediary form does not appear at all during the titration. This case is the one usually encountered in organic dye stuffs. The problem is now: to what magnitude can the degree of overlapping be correlated? This quantity is understood from considering the following chemical process:

$$2 \text{ Mol Intermediate} \rightleftharpoons 1 \text{ Mol Oxidized} + 1 \text{ Mol Reduced.}$$

This formulation symbolizes the fact that there must be established a chemical equilibrium among the three forms. According to the mass action law this equilibrium is fixed by the equation:

$$\frac{(\text{Intermediate})^2}{(\text{Oxidized})\ (\text{Reduced})} = K.$$

The constant K may be called the formation constant of the semiquinone; its reciprocal value may be called the dismutation constant, because this reaction, resembling the Cannizzaro process, is often referred to as dismutation. It is the magnitude of this formation constant which is correlated with the degree of overlapping. This is best shown in the following graph (Fig. 2). When K is very large, say 100,000, no overlapping takes place at all, the two steps are widely separated. As K becomes smaller, the two steps still remain separated but the jump in the middle of the curve is smaller. As K becomes still smaller, the overlapping becomes more manifest, but still in such a way that a kind of jump is visible in the middle of the curve. This is true until K becomes equal to 4. Here the jump disappears, the curve is entirely smooth and has precisely the same form as in an ordinary one-step system such as ferro-ferri-cyanide. However, the two-step nature of the system can be detected during the titration by the fact that a twofold shift in color takes place. When K becomes smaller than 4, the character of the curve is the same, except for the fact that the steepness of the curve is diminished, and as K approaches 0 the steepness is reduced to such an extent that we have to deal now with an ordinary curve of organic dye stuff with no intermediate step at all.

In a two-step system, it is insufficient to speak of "a" normal potential of the system. There are three reversible oxidation-reduction systems within the solution in equilibrium with each other. The first system consists of the reduced and the intermediate form; the second, of the intermediate and the oxidized form; and the third system of the reduced and oxidized form. When the titration has reached that point where the reduced and intermediate forms are present in equal amounts, the potential may be termed the normal potential of the first step, E_1. When the titration has come to the point where the intermediate and the oxidized forms have the same concentration, the potential may be designated as the normal potential of the second step, E_2. At the mid-point of the titration the reduced form will always be present in the same amount as the fully oxidized. The potential at the mid-point of the titration may be termed the middle normal potential, E_m. In any case, there will be:

$$E_m = \frac{E_1 + E_2}{2},$$

and E_m will always be the potential at the mid-point of titration. The problem is now: At which

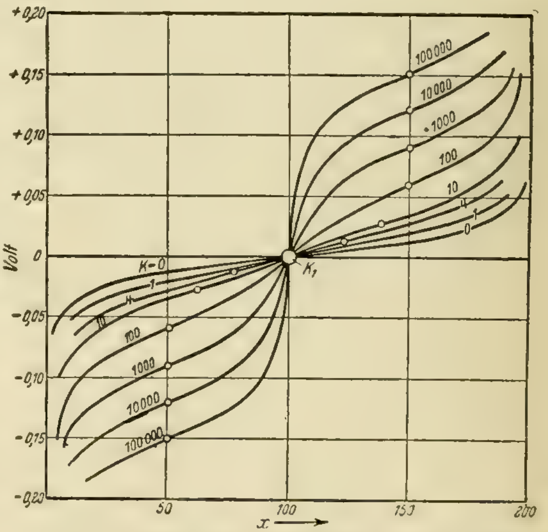


Fig. 2. Electrometric titration curves when the reduced form of a two-stage reversibly oxidizable substance is titrated with an oxidizing agent.

Abscissae: Quantity of added oxidizing agent.
Ordinates: Potentials referred to the potential at half-oxidation ($x = 100$) as zero. Each curve applies for the indicated value of K.

point of the titration will the potential = E_1, or E_2? The answer is easy when no overlapping takes place. Then E_1 is the potential at 25% oxidation, and E_2 that at 75% oxidation. When K becomes smaller, and the overlapping more manifest, the two points of the curve corresponding to E_1 and E_2 are shifted towards the middle and approach each other. When K equals 4, all three normal potentials coincide at 50% oxidation. When K becomes smaller than 4, E_1 shifts over to the right hand side and E_2 to the left hand side; as K approaches 0, E_1 has shifted to the point of 100% oxidation, whereupon obviously E_1 becomes ∞ and in the same way E_2 lies at 0% oxidation and equals $-\infty$. The meaning of this infiniteness is of course that one can no longer speak of a two-step system.

Now, looking again at the above formula which defines K, we have to consider the fact that all of the three substances according to pH may be present in various states of acidic dissociation, and so the value of K will be dependent on pH. Accordingly, as we vary pH in a series of titration experiments, the degree of overlapping in the two steps will vary. For each individual titration curve we can compute the value of E_1, E_2, and E_m by a mathematical analysis of the experimental titration curve. The method of computing these data is too complicated to explain in a lecture, and I shall show only the result for the instance of pyocyanine.

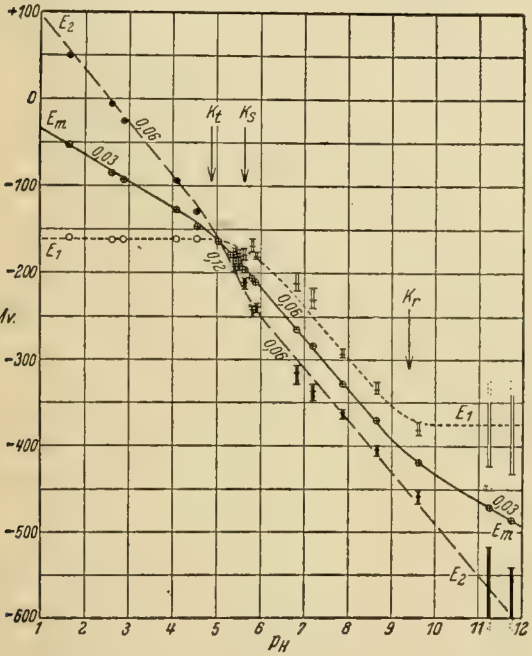


Fig. 3. The three normal potentials of pyocyanine:

E_1 = normal potential of the first (more negative) step.

E_2 = normal potential of the second (more positive) step.

E_m = middle potential. K_t, K_s and K_r are dissociation constants of the oxidized, intermediate and reduced forms respectively.

In Fig. 3 the three normal potentials E_1, E_2, and E_m are plotted against pH for pyocyanine. In order to interpret the slope and the bendings in such curves we have to recall some rules developed by W. M. Clark and Barnett Cohen:

In general such a curve will consist of rectilinear parts connected by smoothly curved bendings. The slope of each rectilinear part is either 0 (the line is horizontal), or, according to circumstances, amounts to .03 Volt or an integral multiple of it, per pH unit. The point of intersection of two adjacent slopes projected on the abscissa indicates a dissociation constant. When the curve becomes steeper with increasing pH, the dissociation constant is of the oxidized form; when it becomes flatter it is the dissociation constant of the reduced form. When the system is a one electron system, each dissociation constant causes a shift of the slope by .06 Volt per pH unit. When the system is a two electron system, the shift amounts to .03 Volt per pH unit.

The normal potential termed E_m belongs to a two electron system, whereas both E_1 and E_2 belong to a one electron system. To give an example: The oxidized form of pyocyanine has a dissociation constant $p^k = 4.9$, at which the color shifts from blue to red. In fact, at pH4.9 there is a bend in the E_2 and in the E_m curve but there is no bend in the E_1 curve because the oxidized form does not belong to the E_1 – system. The shift of this slope amounts to .06 Volt per pH unit in the E_2 system, as it is a one electron system. The corresponding shift in the E_m curve amounts to but .03 Volt, as this is a two electron system.

As a result of the various dissociation constants of the three forms it happens that the three curves not only are not parallel, but even cross each other. The crossing point is the one at which the three potentials equal each other, the formation constant of the semiquinone K being unity. To the right hand side, E_1 is more positive than E_2. This causes a distinct overlapping of the two halves of the individual titration curve. At very high pH values the divergence of E_1 and E_2 becomes very great. The significance of the latter phenomenon is this: when even a small amount of oxidant is added to the reduced form of the dye, the second step of oxidation arises rather than the first, in the same way as in the oxidation of copper the cupric state arises rather than the

cuprous. This is what usually happens on oxidizing the reduced form of a quinoid dye of the regular type.

It is remarkable that for all reversible two-step dyes which as yet are known as occurring in living organisms, the behaviour within the physiological pH range (say from 6 to 8) is such that the two steps are not very widely separated indeed, yet the overlapping is not very great, the formation constant K being, say, between 4 and 1. Therefore, these dyes will in general be present, either within the cells or in the surrounding liquid, to a small but finite and measurable percentage in the form of the semiquinoid. It is impossible to imagine that such an occurrence is a pure chance. Once more, however, I wish to emphasize that the time is not yet mature to utter those speculations on the biological significance which one may have in mind.

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Discussion

Dr. Cohen: What evidence is there as to the molecular weights of these semiquinones?

Dr. Michaelis: Weitz determined molecular weights of some of the Wurster dyes by the boiling-point method. He arrived at the same conclusion about a year prior to me and pointed out that Willstaetter's interpretation of a bimolecular compound was incorrect. Ten years previously, Hantzsch suggested the same idea but had no way of proving it.

Dr. Stiehler: Were the molecular weight determinations made in water?

Dr. Michaelis: No, in some organic solvent.

Dr. Cohen: The existence of free radicals in organic solvents is well-established; but their occurrence in benzene, for instance, is no proof that they can exist in water. The molecular weight of such a compound in benzene may be the same or different in water. The organic free radicals postulated by Dr. Michaelis are apparently stable in water under acid conditions, whereas the known free radicals are highly unstable in this environment. There has been presented a plausible extension of the concept of radicals, and what may turn out to be a useful generalization of oxidation-reduction theory. The idea of this new type of radical should stimulate the organic chemist to search such compounds out and characterize them adequately.

Dr. Michaelis: There are other instances of this in the field of organic chemistry. Similar phenomena have been encountered with triphenylmethyl. It is true that these exist only in organic solvents.

Dr. Barron: In biological systems, we think that the oxidizing catalyst is an electromotively active system with a one-electron transfer, e.g., hemin and its derivatives. These one-step changes are true of a number of compounds found in biological systems.

REVERSIBLE OXIDATION-REDUCTION POTENTIALS IN DYE SYSTEMS

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When a noble metal electrode (such as platinum or gold) is placed in an acid solution of a mixture of ferrous and ferric chlorides it will very quickly assume a stable potential which is determined by the ratio of ferrous to ferric ions. This relation may be written.

$$E_h = E_o - \frac{\mathbf{R}T}{\mathbf{F}} \, ln \frac{(Fe^{++})}{(Fe^{+++})} \quad ..(1)$$

E_h is the observed difference in electromotive force between the electrode and the normal hydrogen electrode; E_o is a constant characteristic for the ferrous-ferric system (the so-called normal potential); **R**, T, and **F** have their customary significances; the parentheses represent concentrations of the corresponding components.

There are a large number of systems organic as well as inorganic which behave in a similar way. Each of them is able to induce on the electrode a reversible potential which is thermodynamically definable. Such systems may be described as electromotively active and easily reversible.

There is another, larger group of oxidation-reduction systems which apparently are electromotively inactive. These fail to impose definable potentials on the electrode. The reasons for these failures are often obscure. In some cases they act as if the proper catalyst were absent; in others, there is evidence that one of the active components may be rapidly destroyed thereby upsetting the equilibrium conditions which are essential to the establishment of significant potentials in an oxidation-reduction cell.

The present discussion will be limited to a consideration of certain simple reversible systems, especially certain groups of organic dyes which can be employed as indicators of oxidation-reduction.

Let us return to the iron system for the elaboration of certain important concepts. (cf. Clark, 1923; Clark and Cohen, 1923).

The oxidation or reduction may be imagined to occur in several different ways. One may consider the oxidation of the ferrous iron to be due to oxygen, or to chlorine or to its electrochemical equivalent, i.e., the removal of an electron. Reversely, the reduction may be imagined as due to hydrogen or its equivalent: the addition of an electron to the ferric ion. These processes may be represented schematically as follows:

$$\xleftarrow{\quad\quad} \text{reduction}$$
$$\text{oxidation} \xrightarrow{\quad\quad}$$
$$\begin{array}{ccccc} 2\,FeO & + & O & \rightleftharpoons & Fe_2O_3 \\ + & & & & + \\ 4\,HCl & & & & 6\,HCl \\ \downarrow & & & & \downarrow \\ 2\,FeCl_2 & + & 2\,Cl & \rightleftharpoons & 2\,FeCl_3 \end{array}$$
$$\begin{array}{ccc} 2\,FeCl_2 + 2\,HCl & \rightleftharpoons & H_2 + 2\,FeCl_3 \\ \downarrow & & \downarrow \\ 2\,Fe^{++} & \rightleftharpoons & 2\,\mathbf{e} + 2\,Fe^{+++} \end{array} \quad (2)$$

It seems quite evident that one of the fundamental parts of an oxidation-reduction process is a transfer of electrons. Whether or not free electrons really exist in aqueous solution, it is convenient from the standpoint of formal treatment and discussion of interrelations to assume that all "electromotively active" oxidation-reduction systems produce a virtual free-electron tension which can be picked up by a suitable electrode.

Then for the reaction (equation 2) involving the electron transfer in the iron system, the equilibrium state may be defined by

$$\frac{(Fe^{+++})\,(\mathbf{e}_S)}{(Fe^{++})} = K_{Fe} \quad ....(3)$$

which gives for the electron transfer tendency in the solution the relation

$$(\mathbf{e}_s) = K_{Fe} \frac{(Fe^{++})}{(Fe^{+++})} \quad ....(4)$$

The electron activity $(\mathbf{e}_m)$ in the noble metal electrode is experimentally a constant and we shall so consider it. Then, for the process occurring at the electrode, the work W required to transfer isothermally one faraday of electrons from activity $(\mathbf{e}_m)$ to activity $(\mathbf{e}_s)$ is

$$W = E\mathbf{F} = \mathbf{R}T \, ln \frac{(\mathbf{e}_m)}{(\mathbf{e}_s)} \quad ....(5)$$

that is, $E = \frac{\mathbf{RT}}{\mathbf{F}} ln\ (\mathbf{e}_m) - \frac{\mathbf{RT}}{\mathbf{F}} ln\ (\mathbf{e}_s)$

$$= \text{Constant} - \frac{\mathbf{RT}}{\mathbf{F}} ln\ (\mathbf{e}_s) \quad ....(6)$$

Substituting in (6) the value for $(\mathbf{e}_s)$ from equation (4), we obtain

$$E = C - \frac{\mathbf{R}T}{\mathbf{F}}\, ln\, K \frac{(Fe^{++})}{(Fe^{+++})}$$

or, when the potential is referred to the normal hydrogen electrode as a standard,

$$E_h = E_o - \frac{\mathbf{R}T}{\mathbf{F}}\, ln \frac{(Fe^{++})}{(Fe^{+++})} \quad \ldots . (7)$$

For the oxidation-reduction in the hydrogen system the reaction may be described as

$$2\,H^+ + 2\,\mathbf{e} \rightleftharpoons H_2$$

and by similar reasoning there is obtained the equation

$$E_h = E_H - \frac{\mathbf{R}T}{\mathbf{F}}\, ln \frac{\sqrt{(H_2)}}{(H^+)}$$

or more familiarly,

$$E_h = E_H - \frac{\mathbf{R}T}{\mathbf{F}}\, ln \frac{\sqrt{P_{H_2}}}{(H^+)} \quad \ldots . (8)$$

This is the general equation for a hydrogen electrode. When $P = 1$ and $(H^+) = 1$, E_H is zero by definition.

For the oxygen system,

$$O_2 + 2\,H^+ + 4\,\mathbf{e} \rightleftharpoons 2\,OH^-$$

$$E_h = E_{O_2} - \frac{\mathbf{R}T}{\mathbf{F}}\, ln \frac{(\overline{OH})}{\sqrt[4]{P_{O_2}}} \quad \ldots (9)$$

For an oxidation-reduction system represented by the dye compounds to be discussed presently, the type reaction is the following (in one or another of its variations):

$$Ox + 2\,\mathbf{e} \rightleftharpoons Red^{--}$$

and,
$$E_h = E_o - \frac{\mathbf{R}T}{2\mathbf{F}}\, ln \frac{(Red^{--})}{(Ox)} \quad \ldots (10)$$

where Ox represents the oxidant, and Red, the reductant. If we plot E_h against percentage reduction, we obtain an S-shaped curve the position of which on the E_h axis depends on the value of E_o which fixes the middle point of the curve, i. e., when the ratio of reductant to oxidant is unity, $E_h = E_o$. The slope of the curve is determined by the value of n, the number of electrons involved in the reaction: it is flatter when $n = 2$ than when $n = 1$. Within any particular system, E_h depends on the ratio of reductant to oxidant.

It is thus possible to express relative oxidation-reduction intensities in terms of electrode potential. One of the problems that has occupied our attention for a number of years is the development of a series of indicators to register differences in oxidation-reduction intensity analogous to those employed in the differentiation of hydrogen ion intensities or activities.

The Participation of Hydrions

Since, as we have seen, the oxidation-reduction reaction involves the virtual transfer of one or more electrons, it follows that the electrical charge on each reactant is susceptible to change, with alteration in hydrion concentration. It follows also that one or both of the reactants can be a cation, an anion or, one of them, a neutral molecule as can be seen from the following type reactions:

$$\overset{-}{Ox} + 2\mathbf{e} \rightleftharpoons \overset{---}{Red}$$

$$Ox + 2\,\mathbf{e} \rightleftharpoons \overset{--}{Red}$$

$$Ox^{++} + 2\,\mathbf{e} \rightleftharpoons Red$$

$$Ox^{+++} + 2\mathbf{e} \rightleftharpoons Red^+$$

It is evident, therefore, that the hydrion concentration must play an important rôle in determining the particular ionic species present. Moreover, the electrode potential which is a measure of the free energy change will measure, *pari passu*, the energy of ionization of the ionized reactants. The simple electrode equation, to be useful experimentally, must therefore be amplified to take into account possible ionizations. That is, it must include the ionization equilibrium constants that may be encountered experimentally.

For example, in the case of the reaction: $Ox^+ + 2\,\mathbf{e} \rightleftharpoons Red^-$, the simple electrode equation for which is

$$E_h = E - \frac{\mathbf{R}T}{2\mathbf{F}}\, ln \frac{(Red^-)}{(Ox^+)} \quad \ldots . (11)$$

the total reductant may be defined as the summation:

$$(S_R) = (Red^-) + (HRed) \ldots . (12)$$

and total oxidant as

$$(S_o) = (Ox^+) + (OxOH) \quad \ldots (13)^*$$

The ionization equilibrium for the reductant is

$$\frac{(H^+)\,(Red^-)}{(HRed)} = K_R \quad \ldots . (14)$$

***—For the sake of simplification, concentrations and activities are considered as equivalent in the present discussion.**

and for the oxidant,

$$\frac{(Ox^+)\,(\overline{OH})}{(OxOH)} = \frac{(Ox^+)\,K_w}{(OxOH)\,(H^+)} = K_o \quad (15)$$

When (14) and (15) are substituted in (12) and (13) we obtain expressions for (Red^-) and (Ox^+) respectively which can be substituted in equation (11) to give

$$E_h = E_o - \frac{RT}{2F}\,ln\,\frac{(S_R)}{(S_o)} + \frac{RT}{2F}\,ln\,\frac{K_R\,(H^+) + (H^+)^2}{K_o\,(H^+) + K_w} \quad \ldots .(16)$$

When (H^+) is kept constant by buffering strongly, the last item in (16) is constant and (16) may be written

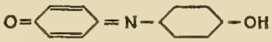
$$E_h = E'_o - \frac{RT}{2F}\,ln\,\frac{(S_R)}{(S_o)} \quad \ldots . (17)$$

This equation contains the quantities that are experimentally determinable, and with it can be constructed the S-shaped titration curves already mentioned.

When ratio of (S_R) to (S_o) is equal to unity, then the last term in equation (16) is the variable which determines the manner in which E'_o varies with change in pH. Each class of oxidation-reduction reaction has its own peculiar variable term which determines the slopes of the E'_o:pH curve.

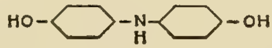
Within the experimentally determinable pH limits of this curve, its analytical geometry shows two important characteristics. (1) When the dissociation constant of an ionic species is altered in the process of oxidation-reduction, the two dissociations (the new and the old) are characterized by bends in the E'_o:pH curve which are centered at the points, pH = pK. (2) Not only are detectable dissociations in the oxidant or reductant determined by the bends in the curve, but such dissociations may be identified as belonging to the oxidant or to the reductant from the change in slope that occurs. Thus, when the change in slope (defining slope as $\frac{-dE}{dpH}$) is negative, the dissociation causing this change is due to an ionic species in the reductant; when the change is positive, the corresponding dissociation belongs to the oxidant. Such dissociations are, of course, also determinable experimentally by acid-base titrations. (Hall, Preisler and Cohen, 1928).

One may summarize the important elements of examination and formulation of a simple oxidation-reduction system by an illustration. Consider the transformation of simple phenol indophenol to its leuco-product



$+ 2e + H_2 \rightleftharpoons$

K_O



K_2 K_1 K_R (K_1 negligibly small)

Its complete electrode equation in the experimentally determinable region of pH is at 30° C.:

$$E_h = E_o - .03\log\frac{(S_R)}{(S_o)} + .03\log\frac{K_R\,K_2\,(H^+) + K_R\,(H^+)^2 + (H^+)^3}{K_o + (H^+)}$$

First Step. Make (H^+) constant. Then the last term of the equation is a constant which, when combined with E_o, is called E'_o. Then

$$E_h = E'_o - .03\log\frac{(S_R)}{(S_o)}$$

Changing the ratio of total reductant (S_R) to total oxidant (S_o) gives the typical sigmoid titration curve.

Second Step. Make $\frac{(S_R)}{(S_o)} = 1$. Then the second term is zero and $E_h = E'_o$. Now vary (H^+). At each value of (H^+) where it equals one of the dissociation constants, K_o, K_R or K_2, there will be a center of inflexion of the curve relating E'_o to (H^+).

Inasmuch as the electrode potential is primarily determined by the percentage reduction (or oxidation) and by the pH, these systems should be visualized as three dimensional surfaces, the coordinates being E_h, pH and percent reduction.

Oxidation-Reduction Indicators

The literature contains numerous references to the use of various substances as indicators of oxidation-reduction change in the solutions containing them. The reduction of litmus in bacterial cultures is a well-known phenomenon which dates back at least as far as Helmholtz (1843). Methylene blue has considerable vogue as an indicator of reduction in a variety of applications which we shall not pause to discuss. It must be pointed out, however, that such applications of

this and other dyes have been based on empirical observations. For the development of systematic indicator theory in the field of oxidation-reduction there is required quantitative information on equilibrium potentials such as we shall now present in brief summary.

Most of the organic compounds which have thus far lent themselves readily to potentiometric measurement of their oxidation-reduction equilibria have the quinonoid structure as a common denominator. Quite a number of such quinonoid compounds can be reversibly reduced to their corresponding benzenoid products; and each oxidant with its reductant sets up reproducible electrode potentials that are thermodynamically definable.

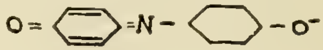
It is rather curious, as well as a challenge to investigation, that the ordinary ethylenic linkage which can often be so readily reduced chemically is not amenable to simple electrometric investigation. In the best known example, the succinate-fumarate system, an enzyme is required to catalyze the equilibrium process; but this is apparently not enough, for a go-between like methylene blue (a quinonoid compound) seems necessary to help establish significant potentials.

Another type of compound that seems to yield significant oxidation-reduction potentials is that included in the class of semiquinones and certain types of "free radicals." These were first studied by Conant, Small and Taylor (1925) and are now being actively investigated by Michaelis; and the elucidation of their peculiar phenomena promises to extend our knowledge of the theory of reversible oxidation-reduction.

We shall now present the essential data for those indicator systems which have been systematically studied and adequately characterized, proceeding along the scale of electrode potential from the electropositive zone to the electronegative. An aid in the visualization ot relations is to imagine a diagram having as abscissae the electrode potentials and as ordinates the pH. On this diagram the line of the hydrogen electrode (at the left) would slope away, decreasing .06 volt (for 30°C.) with each tenfold decrease in hydrion concentration. With this as a baseline the relations of other systems may be gauged. Another fixed base of reference is the line of the theoretical oxygen electrode which runs parallel to that of the hydrogen electrode at a distance of 1.23 volts. The systems which we shall consider lie within these limits. Unfortunately, the indicator systems now known are not evenly spaced between these limits; most of them are crowded more or less near the middle zone and toward the hydrogen electrode.

Indophenol Systems—This group of compounds was studied by Clark, Cohen and coworkers (1923-1928)

The type structure of the oxidant is



The type oxidation-reduction reaction is

$$\overline{Ox} + 2e + H^+ \rightleftharpoons H\overline{Red}$$

The electrode equation (for 30°) is

$$E_h = E_o - 0.03 \log \frac{(S_R)}{(S_o)} + 0.03 \log \frac{K_R K_2 (H^+) + K_R (H^+)^2 + (H^+)^3}{K_o + (H^+)}$$

K_o = dissociation constant of phenolic group in the oxidant

K_R = dissociation constant of same group in the reductant

K_2 = dissociation constant of phenolic group created by reduction

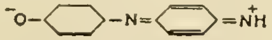
Some thirty different derivatives were studied. The characteristics of a few which seem most suitable for indicator purposes are given in the following table.

Characteristics of Certain Indophenols Useful as Indicators

Name	E'_o at pH 7.0	E_o	pK_o	pK_R	pK_2
Phenol-m-sulfonate indo-2, 6-dibromophenol	0.273	0.6906	7.40	7.12	8.93
m-Chlorophenol indo-2, 6-dichlorophenol	0.254	0.6919	6.16	6.89	9.21
Phenol-o-sulfonate indo-2, 6-dibromophenol	0.242	0.6834	6.07	7.01	10.22
o-Chlorophenol indophenol	0.233	0.6627	7.00	8.44	10.30
2, 6-Dichlorophenol-indophenol	0.217	0.6684	5.70	7.00	10.13
2, 6-Dichlorophenol-indo-o-cresol	0.181	0.6394	5.50	7.10	10.43
1-Naphthol-2-sulfonate indo-2, 6-dichlorophenol	0.119	0.5630	6.14	7.45	9.32

Note: In this and the following tables, E'_o represents the potential at any given pH of a system in which the ratio of oxidant to reductant is unity, E_o is the 'normal' potential, i.e., at pH = 0.

Amino Indophenols—Several members of this group were studied by Cohen and Phillips (1929). These are amphoteric compounds which may be of advantage under certain conditions of indicator application. The type structure of the oxidant is



The type oxidation-reduction reaction may be written

$$\overset{+\,-}{\text{Ox}} + 2\,\mathbf{e} \rightleftharpoons \overset{-\,-}{\text{Red}}$$

The electrode equation is

$$E_h = E_o - .03 \log \frac{(S_R)}{(S_o)} - 0.06\ \text{pH} - .03 \log \left[\frac{K_oK_w}{K_{obl}} + K_o\,(H^+) + (H^+)^2 \right] +$$

$$.03 \log \left[K_RK_2K_3 + K_2K_3\,(H^+) + K_3\,(H^+)^2 + (H^+)^3 \right]$$

K_o = dissociation constant of oxidant's phenolic hydrion

K_{obl} = dissociation constant of oxidant's polar amino group

K_R = dissociation constant of reductant's phenolic hydrion

K_2 = dissociation constant of reductant's 1st amino group

K_3 = dissociation constant of reductant's 2nd amino group

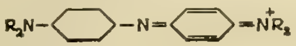
K_w = dissociation product of water

Two compounds in this group may find utility as indicators. These are m-toluylene diamine indophenol and phenol blue. The characteristics of these systems at 30° are given below

System	E'_o at pH 7.0	E_o	pK_o	pK_{obl}	pK_R	pK_2
Phenol blue	0.225	0.677	4.85	high	9.88	5.96
m-Toluylene diamine-indophenol	0.125	0.567	8.07	2.31	10.32	4.96
	$pK_3 = 2.72$; $pK_w = 13.73$					

Indamines—Bindschedler's green and toluylene blue were examined by Phillips, Clark and Cohen (1927).

The type structure is



The type reaction is

$$\overset{+}{\text{Ox}} + 2\,\mathbf{e} \rightleftharpoons \overset{-}{\text{Red}}$$

The electrode equation for 30° is

$$E_h = E_o - .03 \log \frac{(S_R)}{(S_o)} - .06\ \text{pH} - .03 \log \frac{K_{o1}K_{o2}(H^+) + K_{o2}K_w + K_{o1}\,(H^+)^2}{K_2K_3K_4 + K_3K_4(H^+) + K_4(H^+)^2 + (H^+)^3}$$

K_{o1} = dissociation of oxidant's polar group

K_{o2} = dissociation of oxidant's first non-polar group

K_2 = dissociation of reductant's first basic group

K_3 = dissociation of reductant's second basic group

K_4 = dissociation of reductant's third basic group

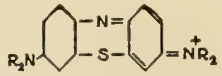
The characteristic constants of these indamines at 30°C. are given below

System	E'_o at pH 7.0	E_o	pK_{o1}	pK_{o2}	pK_2	pK_3	pK_4
Bindschedler's green	0.224	0.680	11.	3.27	6.46	5.10	——
Toluylene blue	0.115	0.601	10.48	3.80	6.56	4.40	2.14

It will be noticed that these systems lie in or near the zone of the indophenols.

Thiazines. Two well-known representatives of this group of dyes, methylene blue and Lauth's violet, were examined by Clark, Cohen and Gibbs (1925).

The type structure is



The type reaction and electrode equation are the same as for the indamines. The characteristic constants of these thiazines at 30° are given below

System	E'_o at pH 7.0	E_o	pK_{o1}	pK_{o2}	pK_2	pK_3
Lauth's violet	0.062	0.563	11.0	low	5.30	5.85
Methylene blue	0.011	0.532	high	low	4.38	4.52

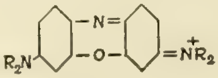
Rapkine, Struyk and Wurmser (1929) have examined the thiazines toluidine blue and azure I. Vellinger (1929) also examined toluidine blue. Although these authors do not report the constants that define these systems, they present curves of the potentials between pH 4 and 9. These show the two systems to lie close to that of methylene blue.

It will be observed that the thiazine systems lie in a zone slightly electronegative to that of toluylene blue.

Oxazines—The oxazine nucleus differs from the thiazine by the substitution of an atom of oxygen for the sulfur of the latter. Four mem-

bers of this group were studied by Cohen and Preisler (1931).

The type structure



The type reaction and electrode equation are the same as those for the methylene blue system. The characteristic constants determined for 30° are as follows

System	E'_o at pH 7.0	E_o	pK_{o1}	pK_{o2}	pK_2	pK_3
Cresyl blue	+.047	0.583	10.7	low	6.3	4.6
Methyl Capri blue	–.061	0.477	high	low	6.10	4.85
Ethyl Capri blue	–.072	0.540	high	low	7.14	6.70
Nile blue - HSO_4	–.122	0.406	9.7	low	6.90	3.92

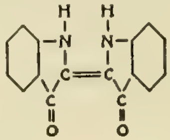
Methyl Capri blue, the analog of methylene blue, is more electronegative than the latter. The oxazine dyes as a group occupy a zone of electrode potential which overlaps the thiazine range and extends a short distance to the electronegative side. Nile blue exhibits very interesting phenomena of reversible molecular aggregation which exert peculiar effects on the equilibrium potentials.

Vellinger (1929) has reported studies on cresyl blue and Nile blue (temperature not given). Rapkine, Struyk and Wurmser (1929) have reported on cresyl blue, Nile blue and cresyl violet at 18°C. Letort (1932) has presented data for 20°C. on the following oxazines: Nile blue 2B, Capri blue, new methylene blue solid cotton blue (Rowe, 910) and muscarine DH. Michaelis (quoting Michaelis and Eagle) reports on the oxazines: brilliant alizarin blue, gallophenin and gallocyanin at 25°. In all the above references the authors present data on potentials in the form of charts or tables which serve as a basis for empirical use as indicators of the dyes named.

It is to be noted, however, that more or less serious discrepancies appear in the data on some dyes studied in different laboratories. While small differences may be accounted for by differences in temperature and technique, a far greater element of error lies in the inadequate identity of the dyes studied. This has two aspects. One is the purity of the material, which is more or less under the control of the experimenter. Another, and more serious danger to the unwary is the acceptance of the manufacturer's name of a dye as being identical in chemical constitution with that having the same name and listed in Schultz's "Farbstofftabellen" or Rowe's "Colour Index." There is no such standardization in the dye industry and we have encountered several instances where unsuspected substituents were present in the compounds obtained on the market.

Indigo sulfonates—This group of acid dyes differs in structure from the basic compounds which we have been discussing. They were studied by Sullivan, Cohen and Clark (1923).

The type structure is



The type reaction is

$$Ox + 2\,e \rightleftharpoons \overset{=}{Red}$$

The electrode equation at 30° is

$$E_h = E_o - 0.03 \log \frac{(S_R)}{(S_o)} + .03 \log [K_1 (H^+) + (H^+)^2]$$

K_1 = first dissociation constant of the group created by reduction.

The characteristic constants at 30°C. for the four indigo sulfonates are given below.

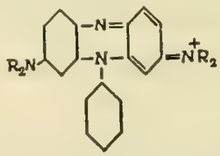
System	E'_o at pH 7.0	E_o	pK_1
Indigo tetrasulfonate	–.046	0.365	6.9
Indigo trisulfonate	–.081	0.332	7.1
Indigo disulfonate	–.125	0.291	7.3
Indigo monosulfonate*	–.159	0.262	7.8

* The monosulfonate is rather poorly soluble when salts are present.

The indigo sulfonates occupy a zone of potential covered by the oxazines and extending somewhat toward the hydrogen electrode.

Safranines—Five of these compounds were examined by Stiehler, Chen and Clark (1933).

The type structure is



The type reaction and the electrode equation are the same as for methylene blue. The characteristic constants at 30° are as follows.

System		E'_o at pH 7.0	E_o	pK_2	pK_3
Phenosafranine	(Rowe 840)	–.252	0.280	4.95	5.8
Tetraethyl "	(Rowe 847)	–.254	0.355	6.4	7.7
Dimethyl "	(Rowe 842)	–.260	0.286	4.9	6.3
Tetramethyl "		–.273	0.288	5.3	6.5
Safranine T	(Rowe 841)	–.289	0.235	4.7	5.7

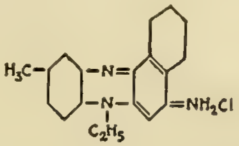
The dye concentration affects the constants to some extent. The above values apply specifically to 0.0001 M solutions.

These systems are all primarily reversible, but the reductants are subject to progressive alterations that must be considered as limiting the reliability of these dyes as oxidation-reduction indicators. Another unfortunate characteristic is that the alterability of the reductants seems to be most marked in the pH range 3 to 7.5.

Neutral Red—This azine is mentioned in passing because it is available in many laboratories and has been employed occasionally as an indicator of oxidation-reduction. However its peculiar irreversibility in the physiological range of pH was reported by Cohen, Chambers and Reznikoff in 1928. This compound was recently examined by Clark and Perkins (1932). The system was found to be primarily reversible and to lie in the region of potentials covered by the safranines. However, it undergoes rather rapid transformation in neutral pH regions to a stable form which does not easily reoxidize. This change is much more pronounced than that occurring in the safranines and rosindulines. We shall therefore omit further consideration of neutral red.

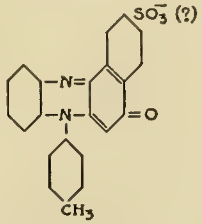
Rosindulines and Rosindones—Six of these were examined by Stiehler (1933). Poor solubility makes four of them unsuitable as indicators. The remaining two, Induline scarlet (Rowe 827) and sulfonated rosindone, seem to be satisfactory. The structures of these are given below

Induline scarlet (Rowe 827)



Sulfonated rosindone (position of sulfonic acid group uncertain)

(C₂H₅)₂N



The type reaction and electrode equation are the same as for the methylene blue system. The characteristic constants at 30° are given below.

System	E'_0 at pH 7.0	E_0	pK_2	pK_3
Rosinduline scarlet (Rowe 827)	–0.296	0.047	4.5	?
Sulfonated rosindone	–0.380	0.25	7.5	9.5

Of particular interest is the fact that these two systems lie close to the hydrogen electrode. Indeed, the potential of the rosindone when 90% reduced at pH 7 equals that of the hydrogen electrode.

Mention should be made at this point of the work of Michaelis (1931) on Rosinduline 2 G (Rowe 830). This compound is a simple unsubstituted monosulfonated rosindone, the E'_0 of which at pH 7 was found by Michaelis to be –0.281. It will be noted that this is about 100 mv. more electropositive than the compound Stiehler examined. Data on some of the azines have been reported by other workers, but unfortunately important details are absent in the published papers. Vellinger (l.c.) gives curves for phenosafranine, safranine (?) and neutral red. Letort (l.c.) gives a curve for phenosafranine which approximately superposes that of Vellinger. They show general agreement with the more detailed data presented by Stiehler, Chen and Clark. Rapkine, Struyk and Wurmser (l.c.) give curves for neutral red, neutral violet and Janus green. The latter is an azo-compound of safranine. On reduction the hydrazo system formed is apparently unstable because the end-product obtained is safranine. The curve given by these authors for Janus green seems to be in fact that representing the system safranine-leuco safranine.

SUMMARY

The foregoing account of oxidation-reduction indicators includes only those well defined systems which are relatively uncomplicated and involve a two-electron transfer in the process of oxidation-reduction. It includes also only those structures in which the oxidant has high tinctorial power, which is important for indicator applications. Consequently no menton has been made of the significant contributions by Conant, Fieser, LaMer, Biilmann and others on various quinones and other compounds of low tinctorial power which give well defined oxidation-reduction potentials.

Certain "meriquinones", "semiquinones" and "free radicals" are known to yield highly colored oxidation products and to give definite equilibrium potentials. The formulation of these systems is now being actively pursued by Michaelis. Relatively few such systems are at present worked out sufficiently to serve as a basis for general application; but more and more of them will no doubt become available as their properties become established.

It will be noted that the adequately defined dye systems at present available do not cover all the possible ranges of potential. There are certain gaps that need to be filled in. Although recent work on the rosindulines and safranines has helped somewhat to fill the gap between the indigo sulfonate zone and the hydrogen electrode, there

is still a need for good stable systems in this region.

Another notable lack is for indicator systems on the positive side of the indophenols toward the oxygen electrode. A number of the simple para- and ortho-quinones lie in this region, but these are weak tinctorially.

It should be pointed out that many of the oxidation-reduction indicators are also acid-base indicators. These two distinct properties should therefore be kept in mind. It is a safe attitude to assume that none of these dyes will remain in aqueous solution indefinitely without decomposition. Indeed, some of them, notably the oxazines, decompose rather rapidly when exposed to water. Consequently difficulties will be avoided if such indicator solutions are made immediately before use.

There appear to be three fields for the application of oxidation-reduction indicators: (1) in the field of inorganic and organic chemistry as an aid in separations, assays and energy studies; (2) in biochemistry, as go-betweens in certain enzymic transformations; (3) in general, as indices of oxidation or reduction intensity in solutions to confirm or substitute for potential measurements, and in solutions where the presence of metallic electrodes is impossible or contraindicated.

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Discussion

Dr. Fricke: Is it possible for you to say more about the actual mechanism whereby the electrons are transferred to the electrode?

Dr. Cohen: This question emphasizes the point that there are two distinct aspects to the theory of oxidation-reduction electrodes. My paper discusses their application to the measurement of free energy change in the transfer of electrons. The matter of mechanism at the electrode is of fundamental importance but the problem is unsolved. One view has it that the noble metal adsorbs atomic hydrogen so that it is in effect a gas electrode. This is conceivable for systems lying close to the hydrogen potential. On the other hand, it is difficult to reconcile this view with the fact that the theoretical hydrogen pressure in equilibrium with a positive indophenol system is of the order of 10^{-20} atmosphere.

With certain types of compounds the equilibrium is reached with remarkable rapidity and potentiometric balance stays constant. In other cases there is a lag, sometimes appreciable—a matter of minutes, hours and even days. If there is decomposition of one of the reactants the time curve of potential may be difficult to interpret. In general, freshly prepared electrode surfaces (e. g. gold plate) reach equilibrium more rapidly than old ones.

Dr. Fricke: Did you in your experience meet with catalytic agencies which are effective in promoting rapid establishment of equilibrium?

Dr. Cohen: The platinized electrode for the $H:H^+$ system is a classical example. The enzyme for the succinate-fumarate system is another.

Dr. Michaelis: The reduction of ferricyanide with hydrogen reaches equilibrium ordinarily in a day, but the addition of a small amount of dye causes it to take place immediately.

Dr. MacInnes: What electrodes did you use for reference in general?

Dr. Cohen: The saturated calomel cell which had been compared with the hydrogen electrode in M/20 potassium acid phthalate. No provision was made for liquid junction potentials.

Dr. Mudd: Were your oxidation-reduction potentials measured by a null-point method?

Dr. Cohen: Yes.

Dr. Mudd: If current were allowed to flow through the detecting circuit, how would that

affect the equilibrium of the oxidation-reduction system?

Dr. Cohen: The experimental concentration cell consists of a calomel half-cell coupled to the oxidation-reduction half-cell. On closed circuit, the whole unit would run down. Thus, if the $Hg:Hg^{+}$ system is the more positive, it will tend to become reduced while the reductant in the other half-cell will be oxidized an equivalent amount. If the $Hg:Hg^{+}$ system is the more negative, it will tend to become oxidized at the expense of the oxidant in the other half-cell. The process will continue until both half-cells reach an equipotential value.

Dr. Mudd: Should not an oxidation-reduction system in the body constitute a source of bioelectric currents?

Dr. Cohen: In a spontaneous oxidation-reduction reaction there is of course a loss in free energy which becomes available for work on the environment. The total amount of it may be expressed in any convenient units, e. g. volt-coulombs, gram-calories, B.T.U., etc. The work may be expended as electricity, heat, light, etc., depending on the particular conditions of the environment. It follows therefore that oxidation-reductions in the body can be a source of bioelectric just as they can be of biothermic or bioluminescent phenomena depending on the path or paths provided by the body for the manifestation of these phenomena.

Dr. Blinks: Can oxidation-reduction potentials exist in liquid junctions or must they be at an electrode?

Dr. Cohen: If a liquid junction were made between two oxidation-reduction systems, a reaction would start at the junction and the free energy liberated would be dissipated by any available path. If a metallic conductor be present, it will filter off the electrons and carry them (producing a negative current of electricity) to the other end of the metallic circuit, provided that (1) there is also available a path whereby negative *ions* can travel in the opposite direction to maintain ionic electroneutrality in the solution, otherwise no current will flow, and (2) the solution at the other end of the conductor has a lower 'electron pressure,' otherwise the current would flow in the opposite direction. It is by this means that electrochemical *potential differences* are established.

Dr. Blinks: Can one get an effective electron transfer that can give rise to the currents you speak of except by means of a metallic surface?

Dr. Cohen: Reversible electron transfer from the reducing to the oxidizing system occurs spontaneously or may be speeded up by a catalyst. In the case of electromotively active systems, a metallic electrode can pick up the 'electron-pressure' and conduct the electron current to a region of lower pressure. Whether effective electron conduction such as this can occur along non-metallic films or membranes remains a question that is unanswered.

Dr. MacInnes: In any purely electrolytic solution the potentials will be due to differences in mobilities and concentrations, and unless there is something analogous to a metal electrode one is not dealing with oxidation-reduction potentials.

Dr. Michaelis: There must be some chemical reaction.

Dr. MacInnes: Just what a potential means if there is no electrode present is worth following up. It would appear to be something like a principle of uncertainty; because anything, (an electrode or a chemical reagent) used as a detector of the potential, changes the latter to some degree.

Dr. Michaelis: Without an electrode there would still be present a level of free energy.

Dr. MacInnes: In the body of the electrolyte one could change the ratio of oxidant to reductant from one region to another and follow the potential change on a probe electrode, but I cannot see that the oxidation-reduction potential at that point would have much meaning except as the electrode is inserted.

Dr. Michaelis: It might be stated in this way: the oxidant-reductant mixtures at each point would be in an equilibrium that determines the potential. The potential manifests itself as an index of the electron pressure of the particular equilibrium present in the vicinity of the electrode.

Dr. MacInnes: The electrons are quite hypothetical.

Dr. Cohen: That seems to be quite true for aqueous solutions; but it is also true that when an oxidant is reduced its electron number is increased, therefore an electron *transfer* must have occurred somehow.

Dr. Müller: Is it possible to have free electrons in a solution?

Dr. Cohen: They are stated to exist in liquid ammonia systems; but in water they could not exist except when attached to something.

Dr. Müller: Is there any reason why electrons cannot exist in water?

Dr. Michaelis: There would be a great tendency for the electrons to reduce hydrogen ions to hydrogen.

Dr. Cohen: The matter of the meaning of oxidation-reduction potentials may be put in another way. They are the index of the energy

state of a system which corresponds to the chemical potential of Gibbs. Whether an electrode is present or not, two reversible oxidation-reduction systems will interact if mixed, and the energy change is measurable in a calorimeter as heat. From this and other thermal data, the oxidation reduction potential may be calculated. The electrode is merely an elegant means for determining the free energy change directly.

The phenomenon of 'oxidation at a distance' should be cited. It is well known that if chlorine be added to iodide solution, the latter will be oxidized and free iodine liberated. Now, put chlorine solution in one vessel and iodide solution in another. Connect the two by a salt bridge plugged with agar to prevent convection and retard diffusion. In the solutions place electrodes and connect them with a metallic conductor (our electron filter). Very soon it will be noted that the region about the electrode in the iodide solution is colored brown from the free iodine liberated. It is as if the iodide ions gave up their negative charges to the electrode which carried the electrons to the region of lower "electron pressure." The discharged iodide ions have become free iodine atoms, while at the other end of the metallic circuit chlorine atoms have been charged (reduced) to become chloride ions. This appears to be a convincing illustration of the phenomenon of electron transfer and of chemical potential as the determinant of oxidation-reduction potential.

Thus it is that the potential of a reversible system is detectable not only by its action on a suitable metallic electrode but also by its action on suitable chemical systems. If the latter are irreversible, interpretation of the result may be rather involved. If they are smoothly reversible and reach quick equilibrium, their properties connected with the equilibrium state may be used as an index of that state; that is, they may serve as indicators. The compounds discussed in my paper are such indicators in which the differences in visible color between oxidant and reductant are utilized.

AN ANALYSIS OF DETERMINATIONS OF INTRACELLULAR REDUCTION POTENTIALS BY MEANS OF INDICATORS[1]

Robert Chambers

We are safe in assuming that the protoplasm of a living cell contains three types of systems which are oxidizable or reducible. They are (a) electromotively active and truly reversible oxidation-reduction systems, (b) sluggishly reversible systems which need to be activated in order to behave reversibly, and (c) systems which are irreversibly affected.

A determination of the reduction potential of such a heterogeneous mixture should be affected primarily by the system present having the most negative potential. It is also conceivable that the presence of irreversible systems which are continually being oxidized during the period of the determination may be affecting the apparent values obtained.

At present we do not know what significance can be ascribed to the values of reduction potential deduced from the behavior of indicators brought into contact with the protoplasm of different cells. The reactions within a living cell presumably never are in a state of equilibrium, and the reducing intensity of one part of a cell may differ widely from that of another. The fact remains that certain definite results have been obtained both under aerobic, and under anaerobic, conditions. These results are fairly constant and reproducible, provided that proper precautions are taken to be sure that the indicator is non-toxic and enters the cell in minimum amounts and does not become too much localized in certain regions. Moreover, the values obtained can be made to shift in the expected direction with varying pH [6] and other conditions, and the rate of reduction of the indicators employed can be changed by agents which presumably affect activating agencies within the cell.

It will suffice to describe the experimental results obtained, the difficulties involved in the technical procedure, and to analyze possible discrepancies in the results obtained by different investigators.

Up to the present time electrometric methods of measuring the potential of protoplasm have been fruitless. It has not been possible to make tips of metal electrodes sufficiently stiff and yet fine enough to penetrate a living cell without occasioning serious injury. It has been possible to make glass microneedles of the proper order of fineness and to convert them into metal electrodes by coating with platinum, or with silver and silver chloride, but they have been unsatisfactory because of their high resistance. Even with ideal electrodes there is still the difficulty of keeping their tips within the protoplasm of a living cell long enough to arrive at some kind of a condition of equilibrium between the electrode and the protoplasm. A microneedle, after being thrust into a starfish egg or an ameba and held stationary for longer than a few seconds, is usually no longer within the protoplasm but is walled off from it by a newly formed plasma-membrane.

The colorimetric method, with the use of oxidation-reduction indicators, has proved much more satisfactory. These indicators, largely prepared and studied by Clark, Cohen and their co-workers, (see Figure), are easily reversible and, if present in sufficiently dilute quantities, readily adjust themselves to the potential of the system into which they are introduced. In general this method of determination parallels the analogous one of determining acid base conditions by means of pH indicators.

Owing to the fact that the potential can be determined only by the maintenance of truly reversible systems, it is imperative that the cell interior be brought into contact not only with the oxidant but also with the reductant of the indicator. Otherwise there would be no way of indicating whether the indicator, which should shift to the potential of the electromotive system present, is behaving in a truly reversible manner.

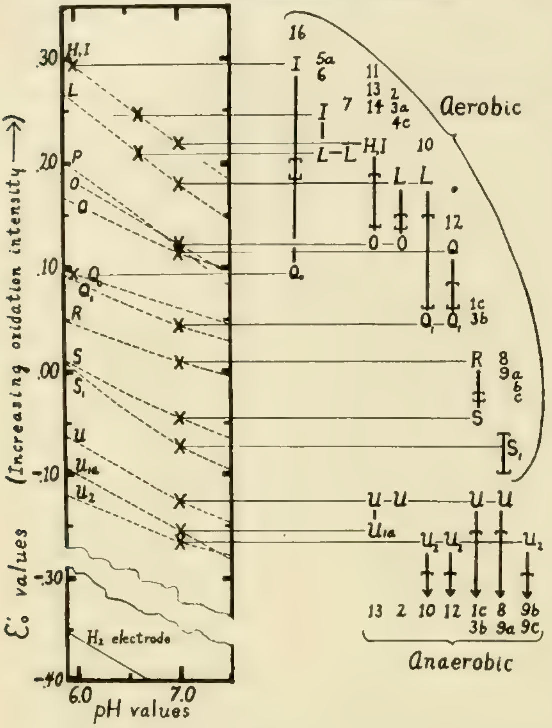
The main advantage of the microinjection method is that it permits the use of indicators to which the living cell is impermeable. Also, owing to the rapidity of the method and to the minute amounts of indicator necessary, there is less danger of upsetting the original equilibria of the cell than by the immersion method.

Its disadvantages are that it cannot be used on many types of cells, either because of their extreme susceptibility to mechanical injury or because of their minute size. Also a quantitative study of capacity and rate factors is not possible because of the inability to inject the same amount into several successive cells, a procedure which is necessary in order to obtain more than a very approximate value. The immersion method is far better suited for the quantitative studies necessary for the determination of capacity and rate factors. However, it is satisfactory only with penetrating indicators.

The micromanipulative technique permits the use of micropipettes having tapering tips as small as ½ a micron in diameter through which aqueous fluids exude easily into such cells as marine

[1] From the Eli Lilly Research Division, Marine Biological Laboratory, Woods Hole, Mass.

Electrode Potential Values Plotted for Cells Under Aerobic and Anaerobic Conditions



Along the ordinate are plotted the potentials (E'o) in volts, with reference to the hydrogen electrode at pH 0.0, exhibited by mixtures, in equal parts, of the oxidized and reduced forms of the reversible indicators. Along the abscissa are plotted the pH values from 6.0 to 7.5 covering the range of the physiological systems investigated.

The curves (lettered according to the scheme in previously published investigations, see Table 1) represent the (E'o values of the indicators used (H to U_2) with variations in pH. Values for the indicators H to U_{1a} are taken from Clark and co-workers; as follows: U_{1a} (35), I (36), Q_0 (37), H, L, O, P, Q, R, S and U (38), Q & S (39). Those for indicator U_2 are taken from Wurmser(40). The crosses on the curves represent the Eo values of the indicators on or next to the borderline of reducibility in the cells. The horizontal lines extend these levels to the right where vertical lines join the levels of the pairs of indicators which delimit the potential value of the particular cell or cells. For example, the figures, 1c and 3b, representing **Amoeba proteus** and **Paracentrotus,** (see Table 2) are placed over a verticle line joining levels at pH 7.0 of R and S, the former being reduced and the latter being oxidized when injected.

The two Ts on the vertical line show the probable outer limits for the aerobic potential on the assumption that R is about 90% reduced and that S is about 75% oxidized.

In a similar manner the anaerobic value for 1c and 3b lies somewhere below the T on the vertical line starting from the indicator U at pH 7.0. The level at which this T is placed is based on the assumption that U is at least 95% reduced, such a condition being assumed since a much larger quantity can be reduced in anaerobiosis than in aerobiosis.

In those cases in which partial reduction is reported only one cross is given. For example, in the case of 8, 9a, 9b and 9c, the reported result is given as a cross on S_1 at pH 7.0. The two Ts on the vertical line at the end of the horizontal extension from this cross mark the probable limit of the potential on the assumption that an indicator reported as parially reduced is in all likelihood not more than 75% oxidized nor more than 90% reduced.

In the case of 16, **Valonia** (see Table 2), the vertical line joining the extensions from I and Q_0 at pH 6.0 represents the range between the two indicators used. The two Ts show the limits of potential calculated by Brooks who claims that I is at least 99.9% reduced and that Q_0 is at least 99.9% oxidized.

ova and protozoa. The most reliable indicators for injection are those which do not penetrate living cells from outside, but, when injected, spread immediately through the cytoplasm.

When injecting a solution of a readily penetrating indicator the experiment may be vitiated if some of the solution is spilled around the cell during the process. The spilled dye may then diffuse into the cell and accumulate there until the possible effects of reduction of a minimum amount are swamped out. Methylene blue, whose reduction potential lies close to the physiological range, is difficult to inject. It tends to coagulate the extraneous coatings of cells so that the pipette clogs before an appreciable amount can be injected into the cytoplasm, where a localized coagulum may also be produced. It tends to cause precipitations and has a strong affinity for certain cell inclusions in which it seems to be more resistant to reduction than when free in solution. Successful injections of indicators of this sort have been made by introducing very dilute solutions in successive small doses so as to permit the cell to exert its reducing capacity on a little at a time.

The indophenols, and especially the sulphonated indicators, have proved to be the best for injection. No coagulation occurs, spilled sulphonated indicators do not penetrate, and when introduced into the cell they diffuse readily and are either reduced or impart to the cytoplasm the homogeneous blue color of the oxident.

In the experiments conducted in the series (1 to 7) precautions were always taken to test the presence of reduced indicators within the cells by subsequently introducing an oxidizing agent, e.g., potassium ferricyanide.

It has been known for some time[8] that dye stuffs which contain sulfonate radicals, do not in general penetrate living cells. Brooks[9] showed this to be true for *Valonia* in regard to (1)naph-

thol(2)sulfonate indolphenol. More recently[3] in studies on the permeability of echinoderm ova to the Clark's series of reversible indicators, it was shown that all those indicators possessing a sulfonate radical (irrespective of their position on the E'o scale) do not penetrate the ova and consequently are not reduced either under aerobic or anaerobic conditions. This has also been found true for starfish spermatozoa[7] and for yeast cells[10].

Another feature to be taken into consideration is the difference in affinity of living cells for various penetrating indicators. Moreover, the accumulation of some of these indicators in a cell is often largely a matter of the difference in the pH of the medium and that of the cell interior. In short, the several indicators which are at our disposal for a colorimetric study of oxidation-reduction potentials vary so greatly in their interactions with biological systems that the greatest precautions must be taken in order to secure adequate interpretations. Many of the observed discrepancies of various investigators may largely be eliminated by a proper consideration of the individual differences among the indicators used.

Special precautions are also necessary if buffered solutions are to be used in which to suspend cells. When a buffer is used the maintenance of a definite pH in the environment does not necessarily mean that corresponding pH conditions are being maintained within the cell. The buffering capacity of the protoplasm of a cell may be temporarily swamped out, and it has been shown that the vacuoles within the cell very readily shift their pH in the presence of penetrating acids[11]. Of the the acids usually used in buffers acetic acid penetrates more readily than phthallic acid which also is able to enter, while citric acid, which is highly polar, and phosphoric acid penetrate much more slowly.

Aside from the special precautions to be kept in mind in detecting the oxidized or reduced state of indicators in the cells, there is still the problem of interpreting results in determining the pH of the cell which must be known before the results from the reactions of the oxidation-reduction indicators can be stated in terms of potential.

Investigators do not agree on the pH value of even the same type of cell[12]. However, most of the publications on such diverse cells as certain protozoa[13, 14], marine ova, both invertebrate [15, 16, 17, 18] and vertebrate[19], several somatic cells [14, 16, 20, 21], and the protoplast of plant cells [22] agree that the pH of the protaplasm of all the cells investigated is within two or three decimal points of neutrality.

The pH values given by Vlès, Reiss and coworkers[23, 24]) as 5.4 – 5.8 have been objected to [15] [4] on the following grounds. In the experiments in which they crushed the eggs[23] the low values were undoubtedly due to the development of an acid of injury, and their spectroscopic experiments[24] deal with pigment changes within granules the pH of which does not necessarily have anything to do with pH of the cytoplasmic mating[18, 11]. Another reported value which seems to be too low is the pH of 6.0 which Rapkine and Wurmser claim for plant cells[25]. This is identical with that obtained by Brooks for the sap of *Valonia* cells[26], while in a recent paper[22] in which the pH of the protoplast and that of the sap have been separately determined, the pH of the protoplast was found to be 6.9± 0.2.

All the determinations of the protoplasmic pH which give values close to neutrality have been made principally by injecting indicators of Clark's series. These indicators diffuse readily through the cytoplasm and give uniform degrees of coloration. They are similar in chemical constitution, all being weak sulfonic acids, and, therefore, are not open to the serious objection of being indiscriminately acidic or basic as is the case with the oxidation-reduction indicators.

Table 1

H Phenol indo-2, 6-dichlorophenol (penetrates)

I Phenol indo-2, 6-dibromophenol (penetrates)

L o-Cresol indo-2, 6-dichlorophenol (penetrates)

O 1-Naphthol-2-sulfonate indophenol (does not penetrate)

P 1-Naphthol-2-sulfonate indo-2, 6-dichlorophenol (does not penetrate)

Q Toluylene blue chloride (penetrates)

Q_0 Thionine (Lauth's violet) (penetrates)

Q_1 Brilliant cresyl blue chloride (penetrates)

R Methylene blue chloride (penetrates)

S K_4 indigo tetrasulfonate (does not penetrate)

S_1 Ethyl Capri blue nitrate (penetrates)

U K_2 indigo disulfonate (does not penetrate)

U_{1a} K_1 indigo monosulfonate (does not penetrate)

U_2 Cresyl violet (penetrates)

Table 1. Indicators used which, by their oxidized or reduced state, show the limits of potential of various cells.

Table 2. Summary of the reported finding in the reducing intensities in certain cells.

	Material	pH: Method of obtaining	pH: Value	Method of obtaining	Limiting Indicators: Most negative ones which are reduced, and most positive which are oxidized under: AEROBIOSIS Red.	AEROBIOSIS Ptly. red.	AEROBIOSIS Oxid.	ANAEROBIOSIS Red.	ANAEROBIOSIS Ptly. red.	ANAEROBIOSIS Oxid.	References	
Protozoa	1a Amoeba proteus (Eur.)	Inj.*	7.6±	Inj.		O			O		N. N. '25, '26[2]	(28, 13)
Protozoa	1b Amoeba dubia (Amer.)	"	7.0± 0.2	"	P	Q R	S	U			C. Ch. P. '28	(1)
Protozoa	1c Amoeba proteus (Eur.)	"	"	"	R		S	U			Ch. C. P. '32	(4)
Protozoa	2 Nyctotherus (Eur.)	"	7.1±	"	L		O		U		N. N. '26[2]	(13)
Marine ova	3a Paracentrotus (Eur.) and Asterias	"	6.6±	"	L		O				N. N. '26[1]	(15)
Marine ova	3b Paracentrotus (Eur.)	"	6.8± 0.2	"	R		S	U			Ch. P. C. '32	(2)
Marine ova	4a Echinocardium (Eur.)	"	6.6±	"	L		O				N. N. '26[1]	(15)
Marine ova	4b Echinocardium (Eur.)	"	6.8± 0.2	"	R						Ch. C. P. '32	(4)
Marine ova	5a Sabellaria (Eur.)	"	6.6±		I		L				N. N. '26[1]	(15)
Marine ova	5b Sabellaria (Eur.)	"	6.8± 0.2	"	L						Ch. C. P. '32	(4)
Marine ova	6 Ophiura (Eur.)	"	6.6±		I		L				N. N. '26[1]	(15)
Marine ova	7 Ascidia (Eur.)	"	6.6±			L					N. N. '26[1]	(15)
Marine ova	8 Echinarachnius (Amer.)	"	6.8± 0.2		R	S_1	S	U			Ch. P. C. '29	(2)
Marine ova	9a Asterias (Amer.)	"	6.8± 0.2	"	R	S_1	S	U			Ch. P. C. '29	(2)
Marine ova	9b Asterias (Amer.)	"	6.8± 0.2	Imm.**	R	S_1		U_2			Ch. B. G. '33	(5)
Marine ova	9c Asterias Cytolyzed	Est. ♯	7.0	"	R	S_1		U_2			Ch. B. G. '33	(5)
	10 Asterias Spermatozoa	" %	7.0	"	L		Q_1	U_2			M. G.	(7)
	11 Mammalian tissues			"	I		O	U			V. J. D. '24	(27)
	12 Yeast	Est. &	7.0	"	Q		Q_1	U_2			B. R.	(10)
	13 Bacteria (luminescent)	" ′	7.0	"	H	K L	O	U		U_{1a}	H. '29	(32)
	14 Salivary gland cells (Chironomus, Calliphora)	Inj.	7.2±	Inj.	I		O				R. W. '28	(13)
Algae	15 Spirogyra	"	6.0±	"	O		R				R. W. '28	(13)
Algae	16 Valonia (Sap)	Est. ″	6.0	Imm.	I		Q_o				Br, '30	(23)

* Value obtained by microinjection of indicators.
** Value obtained by immersion in solutions of indicators.
♯ Eggs cytolyzed in dilute phosphate buffer of pH 7.0.
% Value assumed on basis of value approximated by ova of same species.
& Value assumed.
′ Value assumed.
″ pH of sap determined by use of the glass electrode.

Extracellular Reduction

In all the cells studied by means of microinjection there has been no evidence for the existence of extracellular reduction. In immersion experiments in which indicators known to be non-penetrating have been reported as reduced[26, 27], it is possible that the results are due to the presence of cellular disintegra which possess a pronounced reducing intensity. However, Brooks[25] describes having found that the sap within the vacuole of the *Valonia* cell possesses a definite reducing intensity and Dr. Armstrong (personal communication) reports that the fluid within the brain ventricle of the pipe fish embryo (*Syngnathus fuscus*) reduces the several indolphenols supplied by La Motte. In both cases the extracted fluid shows no reducing intensity.

A similar case appears to be that described by Voegtlin[27] who states that plasma and lymph, in the absence of cellular constituents, possesses no reducing power.

The Intensity Factor of Oxidation-Reduction in the Cell

Needham and Needham were the first to consider the idea of making determinations by injecting indicator solutions into living cells. They obtained varying values of potential for different marine ova (See Table 2—3a, 4a, 5a, 6, 7) the internal pH of which they stated[15] as being uniformly in the neighborhood of 6 6. For the fresh water ameba (Table 2-la,) they obtained[28] a more negative potential, and this they related to their claim that the ameba protoplasm possesses a pH of the more alkaline value of 7.6. Most of their work was carried on under aerobic conditions; they made anaerobic experiments[13] only with *Amoeba proteus* and *Nyctotherus*, (Table 1, 2), the latter a facultative anaerobe. In the ameba they found no difference between the aerobic and anaerobic reduction potential. This led them to believe that the reduction potential of protoplasm of the ameba is poised at a definite level independent of the oxygen tension. With *Nycotherus*, on the other hand, they found an anaerobic value which was definitely more negative than the aerobic.

In later investigations[1-5] special precautions were taken to obtain more strictly anaerobic conditions, and any evidence of intracellular reduction was carefully checked by subsequently injecting an oxidizing agent.

It was found that, under aerobic conditions, such diverse organisms as the fresh water ameba and the several marine ova, both European and American, if their protoplasmic pH be reckoned at 7.0, possess an apparent aerobic potential more negative than +0.011 and probably about –0.072V, [1, 2, 3]. See Figure. With two exceptions all the indicators, which at present number over 30, are consistent in their reactions to indicate the same value. The two exceptions are K_4 indigo tetrasulfonate and ethyl Capri blue. The former, which possesses a less negative E'o potential at pH 7.0 of –0.046V, shows no sign of reduction within the cell, while the latter with a more negative E'o potential of –0.072V appears to be at least partly reduced. Both are relatively non-toxic. A possible explanation is that the tetrasulfonate, being the salt of an acid dye, colors the cell-interior diffusely, while the Capri blue, as a basic salt, tends preferentially to stain certain cytoplasmic granules. If these structures are the seat of more intense reduction it might be expected that ethyl Capri blue would be more readily reduced than would the sulfonate.

Under anaerobic conditions all cells investigated by means of microinjection gave uniform indications of a potential definitely more negative than –0.125V[1, 2, 4], a value which Needham and Needham[13] considered to be the lower limit for *Nyctotherus*. Recently the indicator, cresyl violet, with a still more negative potential of –0.167 at pH 7.0, has been found to be reversibly completely reduced by starfish eggs[5], by starfish spermatozoa[7] and by yeast cells[10]. These values were obtained by observing the decoloration of stained masses of cells in exhausted Thunberg tubes and noting the return of color on the admission of air.

It is of interest to note that a suspension of osmotically cytolyzed starfish eggs gives indications of the same reduction potential as intact eggs, both under aerobic and under anaerobic conditions[5].

In regard to plant cells with large vacuoles filled with sap, Brooks[29, 25] found, by immersion experiments, that the sap, within the intact *Valonia* plant cell, can maintain certain dyes in the reduced condition. If the sap is removed, the reduced dye quickly oxidizes. The sap of *Valonia macrophysa*, according to Brooks, consists of a nearly pure solution of KCl, NaCl and $CaCl_2$ and can have no reducing ability of itself. She maintains that it accepts the oxidized and reduced form of the dye as it passes through the protoplasm. It is surprising that sufficient anaerobic conditions to prevent oxidation of the indicators can be maintained in the voluminous vacuole of the size of a hen's egg and surrounded by a layer of protoplasm not more than 3-10 micra in depth.

In her later paper [25] Brooks made a revision of her earlier data [29] on the value of the aerobic potential by using thionine as an indicator. She considered the reduction potential of the sap to be an index of that of the protoplasm and assumed them to be the same, see Chart

and Table 1. Rapkine and Wurmser[16] found values for the aerobic potential of *Spirogyra* which are somewhat more negative than those of Brooks for *Valonia* and proposed the generalization that the reduction potential of plant cells is more negative than that of animal tissues. However, they were dealing only with the apparent reduction potential in aerobiosis and by methods in which there is considerable doubt as to whether or not the values obtained were that of the sap alone and not of the protoplasm. Moreover, later publications have shown that the reducing intensity of animal cells is even more negative than the values reported by Brooks and by Rapkine.

One of the earlier papers in which a determination of the reduction intensity of cellular tissues was attempted with the use of indicators of known potentials is that of Voegtlin, Johnson and Dyer[27] on mammalian tissues. They made anaerobic experiments on thin sections of tissues in solutions of various indicators down to and including indigo disulfonate (U in Figure), all of which were reduced. An important feature of the work was that the reduction time of the various indicators was found to decrease with an increase in the electrode potential of the indicators.

As can be seen from the figure, the reducibility of the indicators reported by Voegtlin, Johnson and Dyer corresponds to the anaerobic values obtained for other forms. They also injected the indicators into living animals and obtained, in the majority of cases, an aerobic potential which was considerably more positive, since indicator O was not reduced. It is significant that this indicator is a sulfonated compound and might not have been reduced because of its inability to penetrate the cellular tissues. It is also possible that the reason why they secured reduction of all the indicators, including the sulfonated ones with tissue sections under anaerobic conditions, might have been because of the presence of cytolyzed material.

By eliminating the values (see Figure and Table 2) shown by subsequent investigations to be faulty, we are brought to certain conclusions regarding the reducing intensity of the cytoplasm of at least such cells as the ameba and the various marine ova. All tend to possess an apparent aerobic potential which approaches the E'_0 value of –0.072V and an anaerobic potential which is definitely more negative than –0.125V and, in some cases at least, more negative than –0.167V.

Machlis and Green[7], working in my laboratory, have found by the immersion method that starfish spermatozoa do not possess the same reducing intensity as starfish eggs, the indicators which are reduced by the former indicating an apparent aerobic potential more positive than +0.047V. This may possibly be related to the fact that the spermatozoa contain extremely little cytoplasm, their heads consisting almost entirely of nuclear material. Fragmentary results[2] suggest that the nucleus at least of the immature starfish egg, possesses no observable reduction potential. With the very scanty cytoplasm of the spermatozoa it is possible that a capacity factor may influence the observed results on reducing intensity. At any rate no significant conclusions should be drawn from observations which entail only aerobic determinations since, under anaerobiosis, the reducing intensity of the starfish spermatozoa gives indication of being of the same order (viz. $<$–0.167V) as the reduction potential of starfish eggs. No support is given by these experiments to the claim of Joyet Lavergne[30] that male cells possess a more positive potential than female cells.

Conditions Affecting the Reduction Potential

1. Effect of Cytolysis. With properly controlled experiments there is no evident for an increased reduction intensity at the moment of cytolysis. The observations of Needham and Needham[15] indicating that such a phenomenon exists were based on the disappearance of color in an injected egg with the onset of cytolysis. Later experimental evidence[2, 4] offers an alternative explanation that the dye diffuses out of the cytolyzed cell, a view supported by the fact that a subsequent injection of an oxidizing agent shows no return of color which should be the case if the dye were present in its reduced state. Moreover, it has been recently shown [5] that the reduction potential of a mass of osmotically cytolyzed starfish eggs exhibits no difference in its reducing intensity (aerobic or anaerobic) from that of intact eggs.

2. *Effect of variations in intracellular acidity.* It is well known that the potential value of a truly reversible oxidation-reduction dye system is determined by the pH of the system, being more negative with increasing alkalinity, and more positive with increasing acidity. Beck[6] has investigated this point with respect to the effect of variations in intracellular acidity. He found that the aerobic apparent reduction potential of intact living starfish eggs, determined colorimetrically, becomes markedly more negative than normal in the presence of the penetrating base, ammonia, and it becomes markedly more positive than normal in the presence of the penetrating acid, carbon dioxide. For cytolyzed eggs the anaerobic potential shifted in acid solutions to a markedly more positive value. Anaerobic experiments with intact eggs internally acidified were inconclusive. The cytolyzed material reacted equally well to the organic and inorganic bases

and acids used, while intact eggs reacted only to those acids and bases which are known to penetrate living cells.

3. *Effects of Various Reagents on the Intensity Factor.* In the experiments of Machlis and Green[7] on suspensions of starfish spermatozoa it has been possible to show that ethyl urethane renders more positive the apparent aerobic potential of the suspension while cyanide and H_2S render it more negative. On the other hand, the anaerobic potential is unaffected being the same as that of untreated sperm suspensions.

For the lower range of oxidation-reduction potentials there are at present no available indicators which are truly reversible. Hence, there is no way of dealing with possible effects of many reagents, such as narcotics and oxidation inhibitors, on the anaerobic potential.

Machlis and Green found that the anaerobic potential was destroyed on heating the sperm suspension to 100°C., the anaerobic value obtained being of the same order as that of both heat killed and living spermatozoa under aerobic conditions. This finding indicates that the systems responsible for the anaerobic high reducing intensity of normal cells are largely thermolabile.

Rate Factor

All experimental evidence [27, 4, 31, 5] has indicated clearly that the speed of reduction is greatest for the most electropositive indicators and becomes progressively slower as the potential of the indicator approaches that of the cell. It is of interest to note that there are reactions in biological systems which are affected by the E'o value of the indicators. This is shown by Barron and Hoffman[32] who found that the catalytic effect of the oxidation-reduction indicators on oxygen consumption depends on the position of the indicator on the oxidation-reduction potential scale.

Of considerable significance is the fact that cytolysis has a marked retarding effect on the rate of reduction. This has been shown for starfish eggs [5] osmotically cytolyzed by treatment with distilled water and with hypotonic solutions of sodium phosphate (M/15) at pH 7.0. The apparent potential of the system, however, as we have already noted, remains the same as that of intact and living eggs.

Of the narcotics, phenyl urethane saturated in sea water was not found to have any retarding effect, but this may be due to the extreme dilution of the narcotic since we have been able to obtain a definite retardation in the speed of reduction by using the more soluble ethyl urethane in concentration of 3 per cent. It is of interest to note that both phenyl urethane (saturated ethyl urethane (3%), ether (1/100 saturated) and ethyl alcohol ,0.017) completely destroyed the ability of cytolyzed eggs to reduce cresyl blue and methylene blue within the period of observation of three hours.

Potassium cyanide in concentration of N/10, N/20 and N/100 in sea water was not found to have any effect on the rate of anaerobic reduction of cresyl blue or of methylene blue either by intact or by cytolyzed eggs.

For further information on the effects of various reagents and experimental conditions on the rate factor, a review by Ahlgren[33] may be consulted.

The meaning of the experimentally found reduction potential in cells is still very uncertain and it should be realized that the results of the colorimetric method are based on an indiscriminate average of the cytoplasmic mass of the cells investigated.

The findings that the reducing intensity of spermatozoa[7] and of yeast[10] is shifted by narcotics and oxidation inhibitors agrees with the findings of Keilin[34] on the reaction of cytochrome and suggests that the value of the apparent aerobic potential is determined by the relative rates of activity of intracellular respiratory enzymes.

Regarding the anaerobic reduction potential nothing can be stated at present since the experiments in which due precautions have been taken to obtain as strict anaerobic conditions as possible indicate that the most negative value of the anaerobic potential has not yet been determined.

I take this opportunity of expressing my appreciation to Dr. Lyle V. Beck for his assistance and for preparing the figure illustrating the plotted electrode potentials with reference to the cells investigated.

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DISCUSSION

Discussion is omitted because the author includes in his manuscript as presented for publication salient points of the discussion.

Those who took part in the discussion of Dr. Chambers' paper are Dr. Harold Abramson, Barnett Cohen, Hugo Fricke, Stuart Mudd, and Oscar Riddle.

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ITEMS OF INTEREST

Recent visitors to the Laboratory include, Prof. W. J. Crozier of Harvard University, Dr. Robert Hegner of Johns Hopkins University, Prof. and Mrs. Charles J. Lyon of Dartmouth College, Dr. L. Reiner of New York University, Dr. Jules Freund of Cornell Medical College, Prof. Harry A. Charipper of New York University, Dr. Henry G. Barbour of Yale University School of Medicine, and Dr. andMrs. Leslie E. Sutton of Dr. N. V. Sidgwick's laboratory, Oxford. Dr. Sutton is on his way to Pasadena where he will work with Dr. Linus Pauling on a Rockefeller Foundation Fellowship. He plans to study electron diffraction.

Prof. John T. Buchholz of the University of Illinois has recently arrived with his family. He will spend the remainder of the summer at Carnegie Institution here, as guest investigator.

The annual award of John D. Jones Scholarship, formerly made at Columbia University for use at the Biological Laboratory here, has been turned over to the Scholarship Committee of the Laboratory. The stipend of $250 will be given to one applicant, or divided between two or more applicants, as the Committee decides in any given year. Mr. Jones was a founder of the Biological Laboratory. The Scholarship bearing his name was established some years ago by the Wawepex Society.

Professor Harry A. Charriper, Chairman of the Department of Biology at New York University, has completed arrangements whereby the University will have a research table at the Laboratory each year. At present that table is being used by Prof. H. O. Haterius.

Evening lectures, given at the Laboratory this summer, in addition to those already published in The Collecting Net, include:

Dr. Oscar Riddle, Department of Genetics, Carnegie Institution: "Prolactin and Other Anterior Lobe Hormones."

Dr. Stuart Mudd, University of Pennsylvania Medical School: "Infection and Resistance."

Dr. M. Demerec, Carnegie Institution of Washington: "Genes—An Element Essential for the Life of the Cell."

Dr. S. I. Kornhauser, School of Medicine, University of Louisville: "Studies on Anisolabis."

Dr. A. A. Schaeffer, Temple University: "Protoplasmic Organization of Movement."

Dr. H. S. Conard, Grinnell College: "A Criticism of Succession."

Prof. A. M. Banta, Brown University and Carnegie Institution of Washington: "Some Newer Data on Control of Sex in Cladocera."

Carnegie Corporation has just made a contribution of $2500 to the Laboratory in addition to one of equal amount reported in The Collecting Net of July 15th. This completes payment of a special grant of five thousand dollars from the Corporation this year for the purchase of equipment.

Dr. H. O. Haterius of New York University has recently arrived at the Laboratory to spend the rest of the summer.

Dr. John R. Huggins of the University of Pennsylvania has just come to the Laboratory for a few weeks' residence. His family is with him.

END OF COLD SPRING HARBOR SECTION

EYE COLORS IN THE PARASITIC WASP HABROBRACON AND THEIR BEHAVIOR IN MOSAICS AND IN MULTIPLE RECESSIVES

Dr. Anna R. Whiting
Professor in Biology, Pennsylvania College for Women

The eyes of wild-type Habrobracon are black. All mutations are therefore necessarily lighter than wild-type and all are recessive to it. Four independently segregating loci have been involved in eye color mutations. In the orange locus there are four mutant types. These are light-ocelli (o^1), dahlia (o^d), orange (o), and ivory (o^i), forming with type (O), a multiple allelomorphic series of five factors. Dominance is in the order of intensity of pigmentation, type being dominant to all the others and ivory recessive to them. Two different mutations have occurred in the white locus, white (wh) and carrot (wh^c), which with type (Wh) form a triple allelomorphic series. White is partially dominant to carrot since the heterozygote is cream. The two remaining loci have been identified by one mutation each. They are cantaloup (c) and maroon (ma), each recessive to its type allelomorph (C and Ma).

In Habrobracon the second polar nucleus occasionally undergoes division as well as the egg nucleus. If such a binucleate egg is unfertilized and from a mother heterozygous for eye color it may produce a male with mosaic eyes. The behavior of eye colors in relation to each other in mosaics is of considerable interest. Whenever any color in the orange locus is associated in a mosiac with an allelomorph there is no sharp line separating the genetically different regions. Instead, one color shades gradually into the other. An eye, mosaic for black and ivory, shows black pigment at one side, then orange, and finally, at the opposite side of the eye, ivory. An eye mosaic for orange and ivory likewise shows grading. There appears to be diffusion of a chemical substance from the dominant to the recessive region so that some facets genetically recessive show the dominant color.

In striking contrast to this, eye mosaics involving the white locus show a clear cut line between the genetically different tissues. An eye mosaic for black and white or for carrot and white shows each region clearly marked off from the other and obviously autonomous.

The cantaloup locus resembles the white in its behavior in mosaics. No mosaics for maroon have yet been found.

A particularly striking type of interaction is illustrated by eyes mosaic for cantaloup and ivory. Mothers were heterozygous for both ($Cc.Oo^i$) and had black wild-type eyes. There can be no genetically black (OC) tissue in the eyes of the mosaic sons in question since they show cantaloup and ivory regions and would have to have arisen from three different kinds of nuclei if genetically black tissue were present. The cantaloup region is clear cut and marked off sharply from a black band which grades into orange. In the absence of genetically black tissue this black band must be due to the diffusion of a substance from the cantaloup region into the ivory and a physiological reconstitution of the double dominant color.

A black-eyed mother, heterozygous for ivory ($O.o^i$) occasionally produces mosaic sons in which the line of division between black and ivory nuclei does not fall in the eye but in the thorax or abdomen. Gonads of these males are black as shown by breeding tests but the light eyes are not ivory as expected. Instead they are, in most cases, a uniform orange. This likewise appears to be the result of diffusion from the wild-type tissue, possibly the gonads, into the ivory tissue of the eyes. The complete change in color of these eyes, in contrast to the grading of the mosaic eyes, may be explained possibly by supposing that testes or other abdominal tissues develop more rapidly than eyes and produce the darkening chemical substance so early that there is opportunity for complete distribution before the eyes begin to form.

The interactions here described are of interest in view of the opinion held by many that invertebrates, and perhaps more especially insects, differ from the vertebrates in having their tissues autonomous with little chemical or hormonal effect in growth and development.

If light-ocelli (o^1) which was lost several years ago be omitted the mutant eye colors can be combined in forty-eight different ways in the haploid males. Twenty-six of these combinations have been made. Of these nine show pigmentation while seventeen are colorless. The indications are that the remaining twenty-two will likewise be colorless although no predictions can be made with certainty. The intensity of a pigment in a single mutant type has little or no relation to its effect when combined with another. In summarizing these combinations it is clear that every multiple recessive is lighter than the lightest mutant entering into the combination, and the majority of them are colorless. The white resulting from combinations of other mutants is usually a translucent white so that dark underlying tissues show through whereas this is not the case in the single mutant forms white and ivory which are opaque.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 29.)

ANTERIOR PITUITARY-LIKE HORMONE EFFECTS

F. E. Chidester

From the Zoological Laboratory, West Virginia University, and the Marine Biological Laboratory, Woods Hole, Mass.

For a number of years, our experimental work has led us to an appreciation of the close relationship between the endocrine glands and problems of nutrition. Quite recently it has seemed advisable that we bring to the attention of investigators certain correlations that have been emphasized by our converging lines of work, but have apparently been overlooked by others.

The specific points that we wish to stress in this report are (1) the role of iodin in the endocrine chain, and in nutritional conditions favoring reproduction; (2) the role of highly unsaturated hydrocarbons and fatty acids as modifying factors in the action of iodin, calcium and other elements.

Our endocrine studies began in 1912, when, during a summer appointment at the Carnegie Station for Experimental Evolution, Cold Spring Harbor, L. I., we learned that pituitary, thyroid and adrenal extracts apparently inhibited egg laying and somewhat affected body weight of fowls, but possibly exerted a stimulating effect on the reproductive capacity of rabbits. Heavy doses were resisted by pregnant rabbits, but the young were affected in various ways, some exhibiting deformity. During lactation the young were very susceptible to heavy dosage of the mothers.

Later, in 1916 and 1917, we noted the depleting effects in rabbits of nursing large litters, and also receiving thyroid extract, as possibly contributory to paralysis.

In 1918, at the Wistar Institute, we found that heavy doses of thyroid extract or thyroxin (Kendall) apparently induced sterility or resorption of the young in rats. This fact, had been recorded, however, earlier by Gudernatsch (1915) and was reported in 1925 by Pighini. It has been noted by Weichert (1930).

We found that with basic diets (Chidester et al, 1928) rats and rabbits required increased fats and carbohydrates when receiving KI or thyroid extract. Similarly (Chidester et al, 1929) with acid diets, glucose and thyroid together, induced increased weight, and glucose alone apparently hastened maturity in rats. Two grains of thyroid extract daily did not inhibit pregnancies or cause resorption of the young in rats. In chicks and rats, low iodin favored growth including elongation of the bones. This we immediately correlated with the height and body size of mountaineers, living in a low-iodin, goitrous region.

We found that orally administered adrenal cortex, induced prematurity in chicks (Eaton et al, 1929) and in rats, (Chidester et al, 1929). Britton and associates reported similarly in 1931 for mammals. Nice and Schiffer (1931) found that implants of adrenal cortex tissue produced prematurity in young rats.

We had been much impressed by the studies of Crew (1925) who found that thyroid extract rejuvenated aged fowls and caused them to lay eggs that were fertilized by aged cockerels.

Experimental studies since 1927 by our students, Addair, Eaton, Thompson, and Wiles, working with tadpoles, blow flies, mosquitoes and fruit flies, have furnished important information regarding the action of endocrine extracts. Addair learned (1928) that pineal extract apparently caused acceleration of metamorphosis in tadpoles. Other unpublished experiments suggested that adrenal cortex extracts might act similarly. And Eskin (1932) reported that adrenal cortex induced metamorphosis in Axolotl.

It was also shown (Thompson et al., 1928) that orally administered whole pituitary caused increased growth in young rats which was more marked in males.

Wiles (1931), in the most important studies made by us with insects, has shown that adrenal cortex, anterior pituitary and thyroid extract, induced marked acceleration in gonadal development in female Drosophila, but that males were retarded. Similar results to be reported elsewhere (Wiles, Tomblyn, Zucchero, and Chidester) show that iodin induces the same prematurity, thus ruling out possible objection to the nature of the extracts. Males have less of the protective unsaturated fatty acids, but we believe that reduced doses of iodin will act similarly with them. Since 1930 we have studied the influence of extracts of the anterior pituitary hormone on chicks. We found (Chidester and Wiles, 1931), that intravenous injections did not inhibit, but subcutaneous injections stopped ovulations in young laying pullets.

Using the pregnancy urine extract, Antuitrin S, furnished through the courtesy of Dr. Kamm of Parke Davis & Co., we found (Chidester, Ashworth, Ashworth, and Wiles, 1932 a and b) that when it was intravenously injected into young fowls, 7 cases of leg weakness developed in a lot of 14 treated birds. Thyroid extract, furnished in capsules with balancing fatty acids of a wheat preparation, failed to induce such a condition. Our work was checked by a poultryman, who knew of our earlier iodin studies and who

cured leg weakness and protected his flocks with iodized buttermilk. We believe that in our cases the hormone effect was exerted in a similar way to that shown by Loeb, Schockaert, Loeser, Adams, and associates, through the thyroids, when they induced Graves' disease. Notthaft's patient, who took many thyroid tablets for obesity and developed Graves' disease, naturally recurred to our mind. In hyperthyroidism, one finds that osteoporosis may result, since iodin will disperse calcium. Clinical reports support this statement. Mr. Brinton, the poultryman, caused leg weakness in his flock by feeding a diet rich in wheat, but apparently adequate in cod liver oil and fish meal. We believe Mellanby's "toxic factor" of wheat and oats to be the same excess of unsaturated fatty acids that he had noted in 1921 as a cause of goiter.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 15. It will be completed in the reprints.)

THE CARDIAC PARADOXES OF LIMULUS POLYPHEMUS

Iping Chao
Department of Physiology, University of Chicago

It was shown by Libbrecht, (*Arch. internat. de physiol,* 1920, **15**, 446) that when a frog's heart is perfused for some minutes with a potassium-free Ringer's solution and is then again perfused with the normal Ringer, the ventricular contraction is completely inhibited for a short period; by degrees the normal rhythm is resumed. This primary inhibition of the cardiac activity on change from the K-free to the normal Ringer's solution was called by Libbrecht the potassium-paradox (K-P). Libbrecht, (*Arch. internat. de physiol.,* 1921, **16**, 448) also reported a similar primary inhibition on change from a warm to a cold Ringer's solution—the thermo-paradox. More recently Kisch (*Arch. f. exp. Pathol. u. Pharmakol.,* 1930, **148**, 140) found two similar paradoxes in the frog's heart; namely, the calcium-paradox and the strontium-paradox.

Potassium-paradox was recently observed in the Limulus heart by the author (Chao, *Biol. Bull.,* 1933, **64**, 358). This summer a more thorough study of the conditions for its production was carried out, and observations on the calcium-paradox and thermo-paradox were also made; so far the experiments have been performed on the ganglion of the heart only. The results may be summarized as follows:

The K-P is more readily obtained on a second or third repetition than on the first immersion of the ganglion in the K-free solution. When a fresh ganglion is immersed for the first time in a K-free Ringer's solution for ten minutes and is then returned to a normal Ringer's solution the K-P is usually slight or may not appear at all; but on a second trial under similar conditions it invariably appears; and a third trial gives a still more marked result.

The effects designated as paradoxical (the primary and temporary decrease in the rate and amplitude of the heart beat) become more pronounced with increasing duration of the preliminary immersion in the K-free solution. Thus the effect obtained after an immersion in a K-free Ringer's solution for twenty minutes is always greater than that obtained after an immersion of ten minutes.

The K-P is obtained not only on transfer from a K-free to a normal Ringer's solution but also on transfer to a Ringer's solution containing an excess of KCl. It is also obtained on change from a K-poor to a normal Ringer's solution and even on change from a normal Ringer's solution to a Ringer's solution containing a higher concentration of KCl. In other words, the factor determining the K-P is the *sudden change* in the K-concentration, not the absolute concentration of K; and this change must be in the direction of increasing the K-concentration in the second solution.

The presence of NaCl in the K-free solution is absolutely necessary for the production of the K-P; the K-P can be obtained on change from a pure isotonic NaCl solution, but not from a pure isotonic sucrose solution, to an isotonic sucrose solution containing KCl in the concentration normally present in the Ringer's solution. The presence of $CaCl_2$ in the first solution or in the second solution is of only secondary importance—e.g. because of its antagonistic action.

Ca-paradox has been observed in the ganglion of the Limulus heart on changing from a Ca-free to a normal Ringer's solution. The Ca-paradox differs, however, from the K-paradox in that the primary decrease affects only the amplitude and not the rate of the contractions; while in the K-P both rate and amplitude are decreased before the final recovery. In the Ca-paradox also the primary decrease in amplitude is greater, the longer the duration of immersion in the Ca-free Ringer's solution. The effect is obtained only on change from a Ca-free to a normal Ringer's solution (not from a normal to a Ca-free Ringer's solution).

Thermo-paradox can also be obtained in the ganglion of the Limulus heart. Within a certain

range of temperature (up to about 50°C.) the cardiac rhythm increases in rate as the temperature rises. If the temperature is suddenly decreased (e.g. from 40° to 24°C.), both the rate and the amplitude of the beat are temporarily decreased, and in some cases the beat may even be completely arrested for a short period; the beat then returns to the normal rate for the particular temperature. The K-P and Ca-paradox are obtained only on change from lower to higher concentrations of KCl or $CaCl_2$; in an analogous manner the thermo-paradox is obtained only on change from a high to a low, not from a low to a high, temperature.

The fact that the same kind of paradoxical effect (a primary and purely temporary decrease in rate or amplitude, or in both) can be obtained under such different changes of external condition suggests that some general or unitary physiological condition underlies the effect. The nature of that condition is not clearly understood at present.

(The work was partly aided by a scholarship from THE COLLECTING NET, for which the author wishes to express his gratitude).

A RESPONSE OF ARBACIA EGGS TO X-RAYS

DR. P. S. HENSHAW AND DR. D. S. FRANCIS

Biophysical Laboratory, Memorial Hospital, New York, N. Y.

The mode of action of radiation on organisms may be represented as taking place in four steps: 1) irradiation; 2) ionization; 3) chemical change; and 4) biological response. Radiant energy as it moves from its source impinges on atoms and molecules which lie in its path. The result of the interaction of such energy and matter, living or non-living, is that the atoms and molecules lose temporarily some electrons and thus become positive ions. The electrons liberated become attached to other atoms or groups of atoms which thereby become negative ions. The presence of such oppositely charged ions in a compound facilitates a regrouping of the atoms to form new compounds. All of this, (irradiation, ionization, and chemical change) takes place during treatment and is probably entirely completed within a small fraction of a second after the end of irradiation. The biological responses observed are due either directly or indirectly to the chemical changes produced.

From this it is apparent that one of the more important phases of an investigation of the action of radiation on organisms is a consideration of the nature of the chemical changes produced. This, however, is difficult for a number of reasons. Irradiation is the factor controlled and the biological response is the effect observed. But, such a procedure does not indicate how the chemical changes vary with the amount of treatment, nor does it indicate how many types of chemical changes combine to bring about the particular response under consideration. In order to investigate such changes it is necessary to find a response, preferably one which can be measured, which is closely connected with one of the initial changes produced by the radiation. Such a response has been found in the egg of *Arbacia punctulata*.

When Arbacia eggs are exposed to X-rays before fertilization, they are delayed in cleavage—that is, the interval between fertilization and the onset of the first cleavage is prolonged. If the amount of radiation administered is varied, the amount of effect (cleavage delay) is found to vary in the same direction. The effect, therefore, is shown to be a quantitative one. Just how it varies, however, may best be considered by passing directly to the second part of the reaction.

If a given dosage of radiation is administered intermittently, the effect is found to be less than when administered in one continuous treatment. It is possible, in fact, so to space the intervals of treatment that the effect will remain at a constant level. Such experiments show that the effect is not entirely accumulative and that recovery in some form or other takes place.

A more clear demonstration of the recovery process is obtained by allowing time to intervene between treatment and measurement of the effect (i.e. between the end of treatment and fertilization). Here the effect is found to become less as time for recovery is increased. By plotting the amount of effect obtained against the time allowed for recovery, a die-away type of curve is obtained. This shows that the rate of recovery is rapid at first but becomes less as time increases. When various degrees of effect are produced and recovery in each case is determined, a set of recovery curves is obtained which seem to bear a definite relationship to each other. By plotting the data for such a set of curves on a semi-logarithmic scale, parallel straight line curves are found to fit the experimental points as well as any that can be drawn. This indicates that the rate of recovery is the same irrespective of the magnitude of effect produced and that the recovery process at least resembles a reaction which depends on

the concentration of some substance—a monomolecular reaction.

It is a significant fact that recovery is in progress immediately after treatment whether the exposure has been for 5 or 60 minutes to a constant intensity of radiation. From this it appears that recovery is in progress even during treatment. Certain evidence is available which shows this to be the case and which indicates that recovery begins as soon as any effect is produced.

If the same quantity of radiation is delivered to two samples of material in a period of time which is several times longer in one case, the effect is found invariably to be less in the sample which received the longer treatment. The Bunsen Roscoe Law (a constant photo-chemical effect is obtained as long as the product of intensity of radiation and the duration of exposure are kept the same) definitely does not apply here for reasons which are obvious. Recovery takes place during treatment in both cases but relatively more in the longer one. The influence of the time factor during irradiation has been investigated in several ways and essentially the same result was obtained in each instance.

Thus, from the foregoing it appears that X-rays cause a slowing in the rate of cell division in case of the first cleavage in Arbacia eggs and that recovery from the effect begins as soon as any effect is produced. This description gives only the mode of action of a process which is closely associated with an initial effect produced by the X-rays. The nature of the action is not determined as yet.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 22).

STRIATED MUSCLE OF THE LAMELLI-BRANCH MOLLUSC, PECTEN GIBBUS

Herbert L. Eastlick

Assistant in Zoology, Washington University, St. Louis, Missouri

The adductor muscles of Molluscs have long been studied from a histological and cytological standpoint, but doubt yet remains whether or not true striated muscle is present in representatives of this phylum. Although several authors have described a striated-like appearance in molluscan muscle, few have definitely stated that true striated muscle is present. Matthews (1928) states "the general belief seems to be that only smooth muscle fibers are to be found in the molluscs."

The posterior adductor muscle of *Pecten*, the only one present in the adult since the anterior adductor disappears early in the autogeny of the organism, is composed of two very dissimilar types of tissue. Macroscopically, the anterior portion is whitish to faintly brownish in color, soft, almost flabby to the touch, and can easily be teased into fragments. This portion of the muscle is concerned with the rapid closing of the valves and is therefore used in swimming. The posterior part of the adductor is composed of large, coarse, fibers with a glistening appearance. This is the part which keeps the valves closed for relatively long periods once they have been brought together by the action of the anterior portion of the muscle. In this report, a description of the latter only will be given.

Although many structural details can be perceived in teased, unstained material, finer details are portrayed after fixing and staining. In highly magnified stained sections it is seen that the anterior portion of the muscle is composed of parallel myofibrillae which have a definitely striated appearance. The fibrillae vary greatly in size ranging between barely discernible strands to fibrillae having a diameter of 5 to 6 mu; the latter, however, are usually composed of two or three myofibrils so closely joined together that their individuality is not easily perceptible. An individual fibrillum may be analyzed into the following parts which seem to be identical in all respects with similar structures found in vertebrate or other highly differentiated striated muscle: the (Q) disc, which takes an intense stain with haematoxylin, is highly refractive and bi-refringent; the (J) disc, which is about half as tall as (Q), is clear, transparent, isotropic and non-stainable with haematoxylin; and the (H) disc, which appears in the center of the (Q) disc. Besides these discs, two membranes are present, the (Z), or membrane of Krause which bisects the (J) disc, and (M) which bisects the (H) disc. The (Z) membrane does not always have the appearance of a membrane, but is often granular and irregular in outline. Its consistency and appearance undoubtedly varies with different physiological conditions of the muscle, and may be partly due to changes produced by fixation. This membrane has a structure different from that of the other constituents of the fibrillum, as is shown by the fact that it is not readily affected by the section of shrinking or swelling agents. It apparently is quite stable since it maintains its normal width when the fiber is placed in hypotonic solutions which cause swelling at the level of the (Q) disc. Alcohol and certain fixing agents

(*Continued on Page* 346)

The Collecting Net

An independent publication devoted to the scientific work at Woods Hole and Cold Spring Harbor

Edited by Ware Cattell with the assistance of Mary L. Goodson, Rita Guttman, Jean M. Clark, Martin Bronfenbrenner, Margaret Mast and Annaleida S. van't Hoff Cattell.

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THE BEACH SITUATION

Several factors have contributed toward the improvement of the bathing situation on the Bay Shore Beach and the congestion this summer has not been as severe as it was last season. Storms brought about a redistribution of the sandy area; the beach on the southwest side of *the* fence was extended in area and much of the stony ground covered with good sand. Contrariwise, the "private beach" to the Northeast suffered by losing much sand.

Even the offending fence offends less. A section of it was removed at the suggestion of the Department of Public Works of the Commonwealth of Massachusetts!

Although contributions from storm and State have been great, the most fundamental one has come from Dr. O. S. Strong in the form of a gift of the beach rights on his lot adjacent to the fence on the southwest side. These he is reserving "in perpetuity" for both permanent and summer residents of Woods Hole. Dr. Meigs is extending the beach rights of "Lot X" to include the summer as well as the permanent residents of Woods Hole. These bathing privileges are appreciated deeply by members of the laboratory as well as the rest of the community.

THE ELEVENTH NUMBER

An extra number of THE COLLECTING NET will be published about the middle of September in order to accommodate the extra material which has accumulated. Its contents will be of especial interest. Aside from a few papers based upon seminar reports given at the Marine Biological Laboratory, it will contain the following articles:

(1) "Science and Progress" by DR. EDWIN GRANT CONKLIN.

(2) A review of Alfred Korzybski's book "Science and Sanity; an Introduction to Non-artistotelian Systems and General Semantics." by DR. RALPH S. LILLIE.

(3) "The Electric Impedance of Suspensions of Biological Cells," by DR. HUGO FRICKE, Director of the Walter B. James Memorial Laboratory for Biophysics, Cold Spring Harbor Biological Laboratory.

(4) "Electric Conductance of Biological Systems," by DR. KENNETH S. COLE, Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University.

(5) "Phagocytosis," by DR. STUART MUDD, Associate Professor of Bacteriology, School of Medicine, University of Pennsylvania.

(6) "Osmotic Behavior of Red Cells," by DR. ERIC PONDER, Professor of Physiology, New York University.

This copy of THE COLLECTING NET will cost twenty-five cents which will include the mailing charges.

Introducing

DR. VIKTOR HAMBURGER, Fellow of the Rockefeller Foundation working in zoology at the University of Chicago. He arrived in this country last October from the University of Freiburg, Germany, where he held the position of "privat dozent" and taught experimental embryology. At Freiburg, Dr. Hamburger worked on the problem of the physiology of the development of the nervous system in the amphibian and published several papers on the subject in *Roux' Archiv.* On his arrival in this country, he was invited to the laboratory of Dr. Frank R. Lillie at the University of Chicago to investigate similar problems in the embryo of the chicken. Dr. Hamburger came to Woods Hole in July and intends to leave about the middle of September. He will return to Chicago to continue his work there. *R. G.*

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
September 7	7:02	7:27
September 8	7:43	8:13
September 9	8:27	8:59
September 10	9:14	9:50
September 11	10:04	10:44
September 12	10:58	11:42
September 13	11:57	....
September 14	12:41	12:53
September 15	11:35	1:47
September 16	2:25	2:37

In each case the current changes approximately six hours later and runs from the Sound to the Bay. It must be remembered that the schedule printed above is dependent upon the wind. Prolonged winds sometimes cause the turning of the current to occur a half an hour earlier or later than the times given above. The average speed of the current in the hole at maximum is five knots per hour.

ITEMS OF INTEREST

Dr. Frank Blair Hanson, professor of zoology at the Washington University (St. Louis), has been appointed assistant director of the Natural Science Division of the Rockefeller Foundation. He spent last year at St. Louis, but the two preceding years were devoted to work in Paris for the Foundation. Dr. Hanson, who last worked at the Laboratory here in 1918, is visiting Woods Hole for a couple of weeks before taking up his duties at the Rockefeller Center in New York City.

Dr. Warren Weaver, Director of the Natural Science Division of the Rockefeller Foundation, will visit Woods Hole on Wednesday or Thursday of next week.

Dr. A. Hollander is planning to spend part of next winter at the University of Pennsylvania collaborating with Dr. L. V. Heilbrunn in studying the effects of radiation.

Dr. P. W. Whiting has been invited by Dr. C. B. Davenport to spend the coming year at the Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor. Dr. B. R. Speicher will act as research assistant to Dr. Whiting under a grant from the Comittee on Effects of Radiation on Living Organisms, National Research Council.

Dr. George Snell of the University of Texas and now working at the Marine Biological Laboratory, has been appointed assistant professor of genetics at Washington University in St. Louis.

The marriage of Dr. Gardiner Lynn, instructor in biology at Johns Hopkins University, took place in Baltimore on Saturday, September 2. Before her marriage Mrs. Lynn was Miss Harriet Naomi Walker.

The executive committee of the M. B. L. Club has decided to allow members to store their canoes in the club over winter for the nominal sum of $1.00. Further particulars can be obtained from Dr. Charles S. Shoup.

Miss Arlene Johnson, formerly of Oberlin College, has been appointed assistant in zoology at Barnard College for the coming year.

The *Atlantis* sailed today for a ten day trip to the Gulf of Maine with Dr. Redfield in charge. Its further program for the summer has not been planned definitely as yet.

Dr. H. Burr Steinbach of the University of Pennsylvania arrived in Woods Hole on Tuesday. He plans to spend about a month and a half here, working on injury potentials in scallop muscles, before going to the University of Chicago where he will spend the winter.

Mr. Paul Nicoll recently arrived from the Tortugas Laboratory where he has been spending the summer studying primitive chordates.

Miss Edwina Morgulis, daughter of Dr. and Mrs. Serge Morgulis, plans to leave on September 20 for Paris, accompanied by her mother. Miss Morgulis plans another year of study at the Sorbonne.

Dr. Charles D. Snyder, professor of physiology at the Johns Hopkins University, spent Friday night in Woods Hole. He had planned to visit here for a couple of weeks, but was unexpectedly called home because in the recent hurricane a falling tree from Dr. Vincent's lot struck his house caving in a section of it. Rooms on the top floor were the only ones very seriously damaged. Dr. Snyder motored down from the White Mountains with his wife and son who will remain in Woods Hole for a few days.

THE WOODS HOLE LOG

The series of mighty blasts that startled Woods Hole about eight o'clock Monday evening was a call to man the *Acushnet*, preparatory to starting out in search of wreckage. The steamer, *President Hayes*, had reported sighting a square upright post with a steel band attached which was projecting two feet above the water and was apparently attached to submerged wreckage that might be dangerous to shipping.

The ketch, *Nimbus*, which ran aground in the Woods Hole passage shortly before noon on Monday, was hauled off by the Coast Guard cutter 921 early in the evening. Her rudder and bottom were somewhat damaged.

During the height of the strong southwest blow on Labor Day the Coast Guard pulled a thirty-foot catboat from Barnstable off the rocks at Nobska Light.

Over four hundred cases of encephalitis have been reported in the St. Louis epidemic. Three of these cases occurred in families of Washington University faculty members.

STRIATED MUSCLE OF THE LAMELLI-BRANCH MOLLUSC, PECTEN GIBBUS

(*Continued from Page* 343)

often cause shrinkage in other discs, but the width of (Z) remains quite normal.

Fibrillae in which the process of contraction has begun show the presence of the (H) disc. In contraction the (Q) disc separates into two equal parts at the level of the (M) membrane and as the component halves of (Q) migrate toward opposite (Z) membranes, a clear, transparent area, the (H) disc, is formed in the center of the original dark (Q) disc. The (H) disc is very narrow or even invisible in relaxed muscle,but it becomes wider as contraction proceeds and is very evident at the time the contraction band is formed.

It has not been possible to identify the (M) membrane in all fibrillae, since it is extremely faint, and if the staining reaction is not ideal, the contrast between it and (H) is not sufficient to make it apparent to the eye. In properly stained preparations and in stretched fibrils, and in contracted fibrillae in which the contraction bands are sharply differentiated, the (M) line appears as a faint, sometimes granular line bisecting the (H) disc. It is less rigid than (Z) and hence more readily altered by fixatives. It has never been noted in early stages of contraction since the contrast between it and (H) is then very faint.

No doubt exists that the myofibrils, after fixation, are composed of smaller and smaller microscopic units or metafibrils. Such structures are very evident under certain circumstances, but not under others. When present the metafibrils give the myofibril a streaked appearance. Sometimes it is possible to trace these exceedingly minute structures for some distance. There is, of course, a possibility that they are artifact due to fixation, but it is no more logical to interpret them as artifact than to so interpret the fibrillae which can be observed in the living condition in suitable material.

In *Pecten*, however, in contradistinction to ordinary striated muscle, there is no division of the muscle substance into definite fibers. A fiber-like appearance is suggested in longitudinal sections, but transverse sections show that this appearance is due to a considerable ramification of connective tissues which divides the muscle into more or less circumscribed bundles. A true sarcolemma is evidently not present. Moreover, the (Z) membrane is not continuous across an entire muscle bundle but is limited to each myofibril; hence the various discs and membranes are not symmetrically oriented transversely as is ordinarily the case. The fibrillae are surrounded by a minute quantity of sarcoplasm which is non-granular and homogeneous.

Nuclei are diffusely scattered throughout the muscle. Many lie at the inner edge of the connective tissue envelope surrounding a muscle bundle and others appear in the interstices between the fibrillae. The nuclei vary considerably in size and form. Those located between the fibrillae are often very elongate and thin, presumably due to squeezing caused by the contraction of the myofibrillae; but in case the nuclei are free, they are oval or spindle shaped and have an average length of 5 mu and an average width of 3 mu. In the living state they are somewhat larger which shows shrinkage by fixation. Each nucleus contains one or two spherical plasmosomes. These bodies stain intensely with iron haemotoxylin and can always be differentiated from the chromatin aggregations because of their spherical shape and the intense stain they take. The chromatin is scattered throughout the nucleus with the larger aggregations usually located at the periphery in close conjunction with the nuclear wall, although clumps occasionally occur in the center. The innermost area of the nucleus is usually clear and transparent, though it may be faintly colored due to the presence of small granules of chromatin interspersed in the nucleoplasm. In the immediate area about each nucleus a small area of clear sarcoplasm occurs.

Other studies of muscle structure in progress are: (1) a comparison of various invertebrate and vertebrate muscles in an attempt to discover whether or not a "Golgi substance" is present, (2) the existence and location of chondriosomes in striated muscle; (3) changes in fibrillar structure that occur at different phases of contraction and relaxation; and (4) the cytological structure of the smooth muscle of *Pecten*.

This study, and related ones which are in progress, was made possible through the grant of a COLLECTING NET scholarship for which grateful acknowledgement is made.

REFERENCE

Mathews, S. A. 1928. J. Exper. Zool. 51, 209-258.

THE COPEPOD PLANKTON OF THE LAST CRUISE OF THE NON-MAGNETIC SHIP "CARNEGIE"

(*Continued from Page* 309)

plankton surpasses the temperate plankton in number of individuals. The largest number of species were found in the southern Pacific just outside the Humboldt current in the east and north of the Samoan Islands in the west; and in the northern Pacific east of Japan and half way from San Francisco to the Hawaiian Islands.

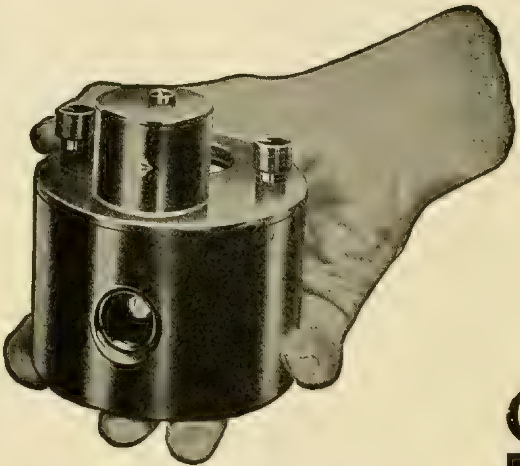
3. Both species and individuals are distributed with extreme irregularity. Of the 260 Pacific species, obtained from 126 stations, only 4 reached a station total of 100, and only 43 were found at more than 50 stations. Of the remainder, 52 were confined to a single station, and 100 others failed to reach a total of more than 25 stations. In short the keynote of the plankton is excessive diversity without even a suggestion of uniformity.

4. The species which make up this plankton are arranged quite definitely in zones or layers. Certain species are confined to the surface, others appear only in the 50 meter net, while a third lot never get nearer the surface than 100 meters. Such stratification is remarkable when we reflect upon the thinness of the upper 100 meters compared with a bottom depth of 3, 4, or 5 thousand meters. Add to this the fact that the nets were non-closing and thus tended to diminish the evidences of stratification and we have excellent proof that the copepods are quite responsive to their environment.

From the data furnished with the plankton—the differences in salinity, density, and hydrogen ion in the upper 100 meters of water are never sufficient to induce any such arrangement of the copepod species. The temperature sometimes varies considerably, especially in the north, and when it does, it no doubt exerts an important influence. But the stratification is even more marked in the tropics, where the temperature varies very little. This suggests that light is the most important factor, not only in controlling diurnal migration but also in determining the depth to which the copepod migrates in the daytime. The abundance or scarcity of food may also be another influence operating along with the temperature and density of light.

(This article is based on a seminar report presented at the Marine Biological Laboratory on August 29).

Cambridge Pot Galvanometer



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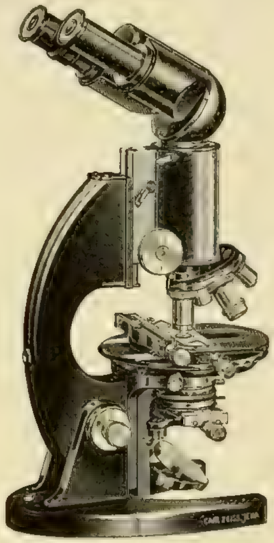

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SCIENCE AND PROGRESS

Dr. Edwin Grant Conklin, *Emeritus Professor of Biology, Princeton University*

I. Fact and Fancy Regarding Progress.

An apology or at least an explanation seems to be demanded for offering a lecture on such a topic as "Science and Progress" in a research institution like the Marine Biological Laboratory. Here we deal necessarily with a high degree of specialization; indeed, specialization in biology has gone so far that many of the persons at the Laboratory complain that they are unable to understand some of the lectures and seminars which are given here. Unfortunately this is a necessary evil. Science does consist in "knowing more and more about less and less", but there is need to turn aside from our specialites from time to time to take a broader view of the course of science in general, and its relationship to human life and welfare. This must be my apology for venturing to speak to you upon such a subject.

I need not comment to this audience upon the content and aims of science, but it may be advisable to consider briefly what is meant by progress. The idea of universal and inevitable progress had its birth only two or three hundred years ago and it is largely a product of biological research. The fact of individual development from a relatively simple germ cell to a complex organism, and more particularly, the concept of organic evolution from simple unicellular forms to the immensely complex animals and plants that people the earth, are largely responsible for this idea of universal and eternal progress. But the biologist knows well enough that such progress usually ends in regression. The individual not only progresses from the germ cell to maturity, but he regresses from this climax to senility and death; and in the same way species have their regression and extinction as well as progress.

Human progress has been along three principle lines, physical, intellectual and social. No one doubts that there has been great progress in all these lines during the past centuries and millenia. The progress of the race even in so short a time as a hundred years is well represented in Chicago's celebration of a "Century of Progress." Material progress is, however, more striking than either intellectual or social progress. At the time when evolution was most in the minds of thinkers, Tennyson wrote:

> *"I doubt not through the ages one increasing purpose runs,*
> *And the minds of men are widened with the process of the suns."*

While we cannot fail to be impressed with the widening of our ideas regarding the universe, natural laws, and the origin and nature of man himself, we may well question whether the intellectual capacities of men are increasing in anything like the same degree as their knowledge.

TABLE OF CONTENTS

The schoolboy of today knows many more things than Socrates or Plato knew, but we may well doubt whether his intellectual *capacity* is superior to that of the greatest men of antiquity. Nevertheless, this vast increase in knowledge which we owe to science has led to wider and truer view of the world. We need only contrast the old view of creation with the new one of evolution, the old superstitions of witchcraft, sorcery and magic with the newer knowledge of medicine and psychiatry to realize how greatly the ideas of men have advanced with the progress of science.

A similar progress is seen in many social relationships. Chief among these are the gradual freeing of mankind from slavery and the substitution of mechanical forces for muscular effort, with the consequent increase of wealth, comforts, luxuries and leisure. Another line of social progress is seen in the growth of democracy, not merely in government, but in all social relationships. Many of us have come to regard democracy as one of the great aims and goals of social progress. Another path of social progress is seen in the increase of populations, the ever larger social units, and especially the growth of internationalism. Science is proverbially international: international congresses of the various sciences have come to be a part of the world's program, and internationalism through science had until recently come to be regarded as one of the most hopeful prospects before the human race. Frequently it was urged that such international relationships must make war impossible, that the human race was on the verge of universal and perpetual peace, that science and progress would go forward together forever. Poets, prophets and seers pictured Utopia, the earthly paradise, "men like gods"—and then came 1914.

II. Revised Concepts of Progress

The world at large now realizes what serious biologists have known for many years, that progress is neither universal nor inevitable, that, indeed, regression is much more common than progress. Evolutionary progress has been brought about only by the elimination of unfit individuals, races and species; social progress is brought about only by the elimination of unfit habits, ideas and emotions.

All the recent progress of mankind has been largely in the realm of environment and it has had but little effect upon the inherited nature of man. Every human being begins life in the valley of the germ cells where his ancestors began; he then climbs to the mountain tops of maturity, only to descend to the valley of death. But society proceeds from generation to generation and progresses from mountain top to mountain top without having to descend in every generation to its primitive beginnings. Thus it happens that the cumulative experience of mankind increases from age to age. Knowledge is ever growing, but not the capacity to know; horse power is multiplied, but not man power; social units are ever becoming larger and more complex, but the human units of which they are composed are born with their primitive traits and emotions.

It is necessary to recognize that life and progress consist in the preservation of a proper balance between contrasting principles and opposing forces. Life itself is a balance between the organism and the environment, between anabolism and katabolism; individual development is a balance between heredity and environment, between differentiation and integration. In the normal human being there is a proper balance between body and mind, emotion and reason; and in normal social development there must be an adequate balance between the individual and the group, between rights and duties. All life is balance and death is loss of balance. Every living creature is like a tight-rope walker over Niagara Gorge.

In one of his lectures in the Old Lecture Hall across the street, Professor Whitman said that "specialization and cooperation are the companion principles of progress." This is illustrated not only in the progress shown in ontogeny and philogeny, but also in the progress of society, and in all of these specialization tends to out-run cooperation. Death and extinction are the results of disintegration. Organisms are not like the Deacon's "One Hoss Shay" that went to pieces

> "*All at once and nothing first,*
> *Just as bubbles do when they burst,*"

but parts fail to cooperate with parts and disintegration results.

Likewise there is balance between instincts and intelligence, emotions and reason. Social cooperation is based largely upon instincts and emotions such as love, sympathy, service within the group, and, on the other hand, fear and hatred for outsiders. In the social insects these are largely inherited and are undisturbed by intelligence and reason. Hence in these organisms we find complete cooperation without compulsion. In man intelligence and reason come in to interfere with instincts and to weaken cooperation. The demand of the individual for freedom, justice and property frequently interferes with the social instincts. "Many men of many minds" make for greater specialization but for less cooperation. Emotions are more uniform and primitive than intelligence and reason, and consequently when it is desired to strengthen cooperation, appeal is made to emotions rather than to reason. In higher animals, passions and emotions are to a large extent held in check by intelligence. The higher nervous cen-

ters frequently act as a brake upon the lower centers. Dr. Bard has found that in his de-cerebrate cats very slight stimuli of an unpleasant sort cause the animals to manifest wild emotions. Lawyers for the defense often speak of a "brain storm", but in view of Dr. Bard's experiments, these might better be called "brainless storms"! In all rational living intelligence must control instincts and emotions.

Emotional behavior is highly infectious. A dog fight sets all the dogs of the neighborhood into a frenzy, and we all know how the mob spirit or war psychology may take possession of an otherwise peaceful and restful society. The only hope for civilization is in learning to control animal passions and emotions by intelligence and reason.

III. Crises in Civilization

We are today in the midst of a great crisis in our civilization. We stand so near these events that we are not able to appreciate properly their great importance, and we are so accustomed to thinking of our civilization as immortal that we cannot easily conceive of its decline and fall. And yet the histories of past civilizations should warn us of the fact that our civilization is no more immortal than that of Egypt, Assyria, Greece and Rome. Indeed there is much to be said in favor of the idea of Spengler that civilizations have their life histories, that they progress from barbarism to feudalism, monarchy, democracy, dictatorship, and finally back to barbarism.

The World War was probably the greatest man-made catastrophe in the history of mankind. It destroyed approximately 20 million lives and 100 billion dollars worth of property, and we are only just now beginning to appreciate the extent of the wreckage which it produced. The "war to end war" has apparently only ended peace, and the war "to make the world safe for democracy" has nearly destroyed democracy. Dictatorships have been established in Russia, Italy, Poland, Austria and Germany, and apparently the United States is moving in the same direction. The fact is that democracy is relatively inefficient in crises which demand instant action, leadership and cooperation. Under such circumstances a wise and beneficient dictatorship is much better than an inefficient democracy. General Wistar used to say that the best government was "autocracy tempered by assasination." I think I should prefer to say that it is "dictatorship tempered by democracy."

In any great social revolution there are inevitable excesses. However much may be accomplished by the great Russian experiment—and I am sure that much is being accomplished—there has been a great loss of freedom of thought, of speech and of science, and in some instances, there has been marked intolerance and cruelty. Yet I have no doubt that the Russian experiment is animated by ideals of progress rather than a desire to destroy that which is high, as Lathrop Stoddard has insisted.

In Italy also, with the marked increase of order, cooperation and prosperity under a wise dictator and a really great man, there has been an end of democracy and the loss of freedom in thought and speech and even in science. We all recall Professor Carlson's plea against attending the international Physiological Congress in Rome because of the loss of scientific freedom in Italy.

The excesses of the German revolution are the more amazing because of the character of the German people. Germany, which has been proverbially the home of science and learning, where freedom to learn and to teach were cardinal principles of the government, has gone back on its liberal past. Dr. Wilhelm Frick, Minister of the Interior, is reported to have said, "Germany's educational system must break wholly with its past to the end that it must strive to produce the man political . . . Service in arms must be the supreme duty and the highest honor." In short, education and science must be directed toward national and political ends rather than toward the development of the individual and the discovery of truth.

The burning of "non-German" books was said to have great "symbolic significance," but its significance is really that it marks the end of intellectual freedom. There is no doubt that some books, both there and here, deserve to be burned, but some of the books that were burned were not of that class. Sinclair Lewis says, "The noblest books of the last twenty years have been burned;" and the letter of our blind and deaf heroine and advocate of international peace, Helen Keller, should be a trumpet call to frenzied patriots to return to reason. She wrote to the student body of Germany: "History has taught you nothing if you think you can kill ideas. Tyrants have tried to do that before, and the ideas have risen up in their might and destroyed them. You can burn my books and the books of the best minds in Europe, but the ideas in them have seeped through a million channels and will continue to quicken other minds. I gave all the royalties of my books for all time to the German soldiers blinded in the World War, with no thought in my heart but love and compassion for the German people. I acknowledge the grievous complications that have led to your intolerance; all the more do I deplore the injustice and unwisdom of passing on to unborn generations the stigma of your deeds."

The worst excess of Hitlerism is its anti-Semitism. Race hatred is far worse than political radicalism, and in the treatment which has been

meted out to leading scientists, scholars, teachers, jurists and patriots of the Jewish race Germany has returned to the methods of the Dark Ages. No doubt much of good is being accomplished by the present revolution in Germany, but it shows a lack of proper balance, and its excesses will be a lasting disgrace to the German people.

The United States is not wholly immune to this infection which is sweeping through the rest of the world. A cartoon in the *Saturday Evening Post* for last August 15 represents the typical "moronic" American looking at the processions following Stalin, Mussolini and Hitler, and saying, "I would like to see them try that on me." Well, it is being tried on us. We also are trying a limited dictatorship. The "rugged individualism" of the last Administration proved to be a failure, and the "enlightened self interest" so much advocated by some philosophers and ethical teachers, has been shown to be inadequate in so complex a society as our own. These shibboleths do not enable us to cope with the conflictions of race and class, of gangsters versus society, and, worse still, of dishonesty and selfishness of great promoters, bankers and public officials. The mottoes of business: "Let the buyer beware," "No profits—nothing doing," "Every man for himself and the devil take the hindmost," show a loss of social morale and mark the collapse of social cooperation.

In this great crisis, we may well ask, is democracy feasible or desirable? Can we attain cooperation without compulsion? Here as everywhere a proper balance between opposing forces is necessary. Balance between the individual and society, between freedom and restriction, persuasion and compulsion, reason and emotion.

IV. Causes and Cures

We need a real scientific diagnosis of the diseases of society before any proper treatment is possible, just as modern medicine has advanced by a study of the cause of diseases and has attempted to treat them by striking at the causes. Many humanists attribute the ills of modern society to science. I need only refer to the address of Woodrow Wilson at Princeton on the "Bankruptcy of Science," or the recent articles of Dean Gauss on the "Threat of Science." There is no doubt that science has greatly increased social specialization without any corresponding increase in cooperation. In short science has not changed human nature, and that is what many of the humanists seem to think that it should have done. The Bishop of Ripon in an address before the British Association for the Advancement of Science in 1927 proposed that science declare a moratorium for ten years until human nature could catch up with increasing knowledge. But apart from the impossibility of stopping scientific investigation, it would take not ten years, but ten centuries or more to change fundamental human nature. To make conduct conform with knowledge is the old, old problem with which education, ethics and religion have always tried to deal. As Shakespeare says, "If to do were as easy to know what were good to do, chapels had been churches and poor men's cottages, princes' palaces;" or in the more pithy saying of Mark Twain, "To be good is noble, but to tell others to be good is noble and no trouble."

Science is knowledge and society is not suffering from too much knowledge, but from the failure to apply that knowledge to social conditions. But while science is not the cause of social disorders, it has greatly augmented their danger. It has put high-powered automobiles and machine guns into the hands of gangsters and criminals. Professor Soddy, inspiration of the late lamented technocrats, has said that "The advance of physical science will be a menace to civilization if our present low social standards persist." Sir Oliver Lodge says, "The atrocities of the World War were wrought with molecular forces—we are not yet fit to handle the much greater atomic forces"; and Raymond Fosdick in his book, "The Old Savage in the New Civilization", asks, "Can the old savage be trusted with the forces he has let loose?"

Although science has increased the dangers to society, it has also increased the means of meeting these dangers. It has shown that men and society are the products of heredity and environment, and it has pointed out how to improve both of these factors. It has shown that social disorders are not so much the results of bad heredity as of bad education, and it has enormously amplified the possibilities of education. Indeed education is the chief hope of human progress, but it must be through liberal, ethical education and not mere propaganda. Unlike the announced new German system, it must glorify peace rather than war, sympathy rather than hate, humanity rather than nationalism. We often hear it said that you cannot change human nature, and that, therefore, wars will never cease. It is true that we cannot change human nature, that is, inherited human traits, except by the process of eugenics; but in the present crisis, the world cannot wait for the slow improvement of human nature by this means. We must rather depend upon the development of the good capacities which we already have rather than upon the development through heredity of new capacities. The disappearance of cannibalism, human sacrifice, polygamy (to a certain extent), the burning of heretics, the torture of wit-

(*Continued on Page* 404)

THE BIOLOGICAL LABORATORY
COLD SPRING HARBOR

THE ELECTRIC IMPEDANCE OF SUSPENSIONS OF BIOLOGICAL CELLS

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The first measurements of the electric resistance of living cells were made in the middle of the foregoing century. Such well-known physicists as Peltier, Weber, v. Humbolt, and Lenz worked in this field. A large amount of work was done by the noted physiologist du Bois Reymond, who summarized the work done previous to 1850, in his "Untersuchungen über tierische Elektrizitat."

The fundamental fact was brought out, that a living cell becomes polarized when an electric current passes through it, the resistance of living matter being, in consequence, high when a direct current, or a current of low frequency, is employed, but decreasing when the frequency is increased. Particular mention should be made of the classical researches of Osterhout[1] on the influence of sodium and calcium ions on the resistance of *Laminaria*. Earlier investigations were handicapped by the experimental difficulties of producing alternating currents over a wide range of frequencies. This difficulty was overcome by the introduction of the audion oscillator, which initiated a period of considerable progress.

The following report chiefly concerns our own work. Two different methods of measurement are used. Up to 2×10^6 cycles, measurements are made with a resistance-capacitance bridge as earlier described[2]. From 2×10^6 to 16×10^6 cycles, a reasonance method is used. In both cases, a substitution method is employed; the electrolytic cell containing the biological material, being replaced by a similar cell containing a salt solution of the same resistance, and connected in parallel to a variable air condenser. One electrode of the comparison cell is mounted on a micrometer screw, which allows a fine adjustment of the resistance to be made. The function of the bridge, or the resonance apparatus, thus is solely to indicate the existence of electrical equivalence. The equivalent resistance and parellel capacitance are termed R and C, per centimeter cube of suspension.

For theoretical study, we may take, as a simplified model, a suspension of conducting particles, at the surface of each of which polarization of the electric current takes place. Consider first the conductivity of a suspension of homogeneous, non-polarizable spheres. The theoretical solution of this problem was given by Maxwell, in his "Treatise on Electricity and Magnetism." He derived the well-known formula:

$$\frac{r_1/r - 1}{r_1/r + 2} = \rho \frac{r_1/r_2 - 1}{r_1/r_2 + 2} \quad (1)$$

where r is the specific resistance of the suspension; r_1 is the specific resistance of the suspending liquid; r_2 is the specific resistance of the suspended particle; and ρ is the volume concentration of suspended material, which in the field of conductivity is usually referred to as Maxwell's formula, but which is known under other names in other departments of physics, such as the Lorenz-Lorenz formula, the Clausius-Mossotti formula, or the Poisson formula.

We have tested this formula over a wide range of volume concentrations by working with cream[3]. A heavy cream was obtained by separating raw, fresh milk in a De Laval cream separator. The conductivity of this cream, of the skimmed milk, and of various mixtures of the two were measured. Table 1 shows the result of such a series. One column gives the volume per cent of the fat particles calculated from Maxwell's formula, which in this case reads:

Table I. Test of Maxwell's formula for the conductivity of a suspension of spheres.

	Volume concentration percent	
	Calc. from resistances	Calc. from dilution factors
Primary cream	62.73 ± .12	62.93 ± .19
Dilution No. 1	40.40 ± .08	40.44 ± .12
Dilution No. 2	22.49 ± .06	Standard

$$\frac{r_1}{r} = \frac{1 - \rho}{1 + 1/2\,\rho} \quad (2)$$

where r is the resistance of cream; r_1 is the resistance of skimmed milk; and ρ is the fat content of the cream. The next column shows the rela-

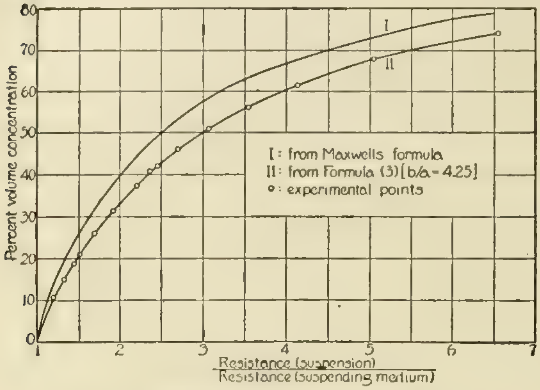

Fig. 1. Resistance of suspensions of red corpuscles of different concentrations.

tive volume concentration calculated from the dilution factor.

Much work has been done on the conductivity of blood. The red corpuscle is a non-conductor, and therefore the conductivity of blood is lower than the conductivity of the serum.

How well does Maxwell's formula apply to a suspension of red corpuscles? In Fig. 1 are plotted experimental data obtained with red corpuscles of a dog[4]. The ordinates indicate volume concentration, and the abscissae, the ratio of resistance of corpuscle-suspension to that of serum. The upper curve is calculated from Maxwell's formula. The reason for the deviation is the non-spherical form of the corpuscles.

It is a fairly simple matter to extend Maxwell's formula to the case of a suspension of spheroids. The following formula is obtained[3]:

$$\frac{r_1/r - 1}{r_1/r + x} = \rho \frac{r_1/r_2 - 1}{r_1/r_2 + x} \qquad (3)$$

where x depends on r_1/r_2 and on the axis ratio a/b of the spheroid, but is independent of the volume concentration. x is shown graphically in Figs. 2 and 3 for the oblate spheroid and the prolate spheroid, respectively. When $a/b = 1$, we have $x = 2$, which is the Maxwell formula.

The curve **II** in Fig. 1, has been calculated from formula (3), using $a/b = 1/4.25$, which gives $x = +1.05$. The agreement is very satisfactory; in fact, it is rather surprising that the agreement is so good at the highest volume concentrations, where the corpuscles are packed far beyond the limit set by the total packing factor, indicating that deformation of the cells must have taken place.

As an absolute method for determining the red corpuscle volume, the conductivity method is not wholly satisfactory for mammals, because of the particular form of the corpuscles. For the lower orders for which the form is ellipsoidal, the method should be able to give results of the highest exactness. Ponder's observation[5] that mammalian corpuscles can be made spherical by adding lecithin, gives a possibility for using the method for exact work with these corpuscles, and we have used it in some preliminary investigations on swelling[6]. The greatest practical difficulty appears to be that of securing perfectly homogeneous suspensions.

The conductivity method may also be useful for the practical determination of the fat content of milk and cream, although no attempt of so using it has been made as yet. Other possible fields of practical application are the determination of rubber in latex, and the analysis of soils

Table 2. Conductivity of suspensions of sand.

r/r_1 (obs.)	ρ (obs.) (percent)	x (calculated)
1.190	10	1.410
1.426	20	1.420
1.734	30	1.405
2.156	40	1.367
2.715	50	1.400
3.613	60	1.35
	Average value	1.392

Table 2 shows some measurements of the conductivity of suspensions of sand, which were made in Wilhelm Ostwald's laboratory many years ago[7]. The suspensions were made by adding the sand to hot salt solutions containing 3 percent gelatine, and cooling this mixture to gelatination under continuous rotation. The concentrations vary from 10 per cent to 60 per cent. We have applied formula (3) to these measurements, for each concentration calculating x, using the

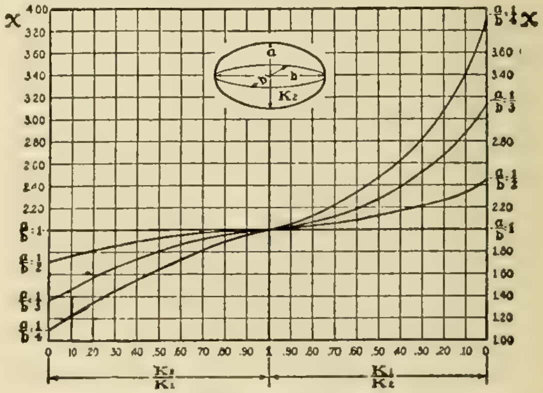

Fig. 2. Graphical representation of x for the case of the oblate spheroid.

experimental values of r/r_1 and ρ. The values of x stay perfectly constant. As x is smaller than 2, the sand particles must have been flat. The average ratio of a/b is about 1/3.

For a current of low frequency most living cells are nonconductors, due to polarization at their surface. In order to obtain the true interior conductivity of the cell, a current of such a high frequency must be used that the impedance at the surface is reduced to zero. This is illustrated by the resistance measurements in Table (3)*, which are for a suspension of red corpuscles of rabbit,

Table 3. Impedance Measurements on a Suspension of Lecithinated Rabbit Corpuscles

Kilocycles per sec.	R (ohms)	C ($\mu\mu$F)	C_s' ($\mu\mu$F) (Eq. 12)	x	$R_i + R_s'$ (ohms) (Eq. 13)	R_s' (ohms) (Eq. 14)	R_i (ohms)
.5	577	(282)*					
1.0	577	(303)*					
8.0	576	(302)*					
16.0	575	298					
32.0	575	292		$.03_0$			
64.0	574	285		$.03_5$			
256	557	273	277		290	136	154
512	524	259	270	$.03_5$	237	66	171
1024	441	232	263	$.03_5$	214	34	180
2048	316	188	256	$.03_5$	183	17	166
8200	150	45.4	252		168	4	164
16400	132	13.8	249		161	2	159
∞	125 (extrapolated)						

* Inaccurate due to electrode polarization.

using currents of different frequencies up to 16×10^6 cycles. Extrapolating to infinite frequency, a resistance of 125 ohms is obtained, and using formula (3) with this value of the resistance, the interior conductivity of the corpuscle may be calculated. We obtain a value of 150 ohms at 20°C, which is approximately twice that of the serum. This high value is mainly accounted for by the hemoglobin contents of the corpuscle.

Now consider a suspension of conducting spheroids, each covered with a thin nonconducting membrane. The specific capacity of the suspension at low frequency is expressed by the following formula[8]:

$$C = \alpha\left(1 - \frac{r_1}{r}\right) q\ C_o \qquad (4)$$

where r is the Conductivity of the suspension; r_1 the Conductivity of the suspending medium;

*—For the purpose of the later discussion, data are shown for corpuscles which have been made spherical by means of lecithin.

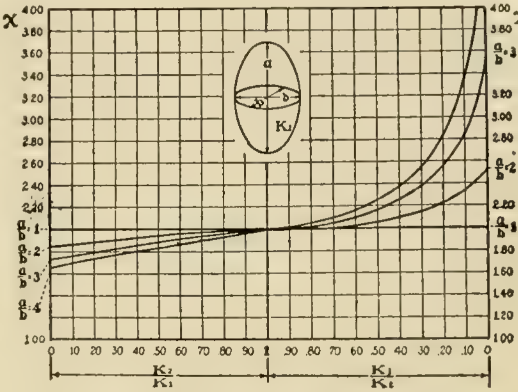

Fig. 3. Graphical representation of x for the case of the prolate spheroid.

q the major axis of the spheroid and C_o the static capacity of one sq. cm. of the membrane α depends on the axis ratio of the spheroid, and its values are shown in Table 4.

Table 4. Values of α as a function of b/a and of a/b.

b/a	1	2	3	4	∞
α	1.50	1.30	1.27	1.28	1.65
a/b	1	2	3	4	∞
α	1.50	1.03	0.94	0.94	(0.118 x a/b)

Axis of spheroid a, b, b,

We may write formula (4) as follows:

$$C = C_{100pc}\,(1 - r_1/r) \qquad (5)$$

$$\text{with } C_{100pc} = \alpha\, q\, C_o \qquad (6)$$

where C_{100pc} is a constant, which may be considered as the capacity of a 100 percent suspension. This formula shows that the capacity varies as $1 - r_1/r$, in its dependence on the volume concentration. In this form, the formula is independent of the form of the spheroid, and may be applied to a suspension of particles of any form.

This formula has been tested by measuring the capacity of suspensions of red corpuscles of different concentrations. Table 5 shows the results of a series of such measurements[2]. By formula (5), C_{100pc} is calculated from the observed values of the capacity C. The values of C_{100pc} are constant from 21 percent to 84 percent concentration.

Consider, finally, a suspension of polarizable homogeneous cells. The impedance of the cell surface may be represented as a capacity, C_s in series with a resistance, R_s. The power factor $m = C_s\, \omega\, R_s$.

Consider that part of the current through the suspension which passes through the interior of

Table 5. Capacity of suspensions of red corpuscles of dog in own serum.

No. of experiment	Volume concentration calculated from resistance	Resistance (r) (ohms)	Capacity (C) mmf	Capacity (C_{100}) for 100 percent concentration (calculated)	Origin
	percent				
I	83.9	931.	374	411	Concentrated by centrifugation from original blood.
II	21.0	126.9	129	385	From 83.9 percent suspension by dilution.
III	72.0	498.	343	411	From 83.9 percent and 21. percent suspensions.
IV	47.5	230.2	237	374	From 72.0 percent suspension by dilution.
V	60.2	329.	286	385	From 83.9 percent and 47.5 percent suspensions.

Resistance of serum: 84.25 ohms.

the cells. At low frequencies, the resistance of the homogeneous liquid, through which this current passes, is inappreciable compared with the impedance at the cell surface. In this case, the suspension may be represented by diagram (A) in Fig. 4, in which R_o represents the resistance of

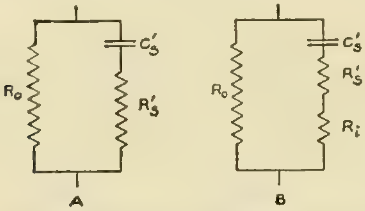

Fig. 4. Diagrammatic representations of a cell suspension.

the intercellular liquid, and C'_s and R'_s the cell surface. The relation of C'_s to C_s is represented by a formula similar to (4):

$$C'_s = a\left(1 - \frac{r_1}{r}\right) q\, C_s \qquad r = R_o$$

R'_s to R_s is determined by

$$m = C_s\,\omega\,R_s = C'_s\,\omega\,R'_s$$

It should be noted that the relatioship of C'_s and R'_s to C_s and R_s only involves the form and size of the cells, and the volume concentration of the suspension, but not the frequency of the electric current. The relation of C'_s and R'_s to the observed values C and R is given by:

$$C = \frac{C'_s}{1 + m^2} \qquad (7)$$

$$1/R = 1/R_o + C\,\omega\,R \quad (8) \qquad m = C'_s\,\omega\,R'_s$$

$$C'_s = C\left(1 + \frac{(1/R - 1/R_o)^2}{(C\,\omega)^2}\right) \qquad (9)$$

$$m = (1/R - 1/R_o)/C\omega \qquad (10)$$

When $C\,\omega\,m$ is small:

$$\triangle R = R - R_o = C\,\omega\,m\,R^2 \qquad (11)$$

These formulae allow the calculation of C'_s and m. R_o is the resistance obtained at low frequencies.

Turn now to the calculation for frequencies so high that the impedance of the cell surface is not very large, compared to the resistance of the homogeneous phases. For a homogeneous suspension of spherical cells, the suspension may be represented by diagram (B)* in Fig. 4 [9]. R_i represents the sum of the resistances of the inter-, and intra-cellular liquid, transversed by that part of the current which passes through the interior of the cells. The following equations hold:

$$C'_s = C\left(1 + \frac{\left(\frac{1}{R} - \frac{1}{R_o}\right)^2}{(C\,\omega)^2}\right) \qquad (12)$$

$$R'_s + R_i = \frac{\frac{1}{R} - \frac{1}{R_o}}{C\,C'_s\,\omega^2} \qquad (13)$$

$$m = C'_s\,\omega\,R'_s\ . \qquad (14)$$

*—No account has been taken in diagram (B) of the static capacitances derived from the homogeneous parts of the suspension. The influence of these capacitances is comparatively small under a frequency of a few million cycles, but plays a dominant role at higher frequencies.

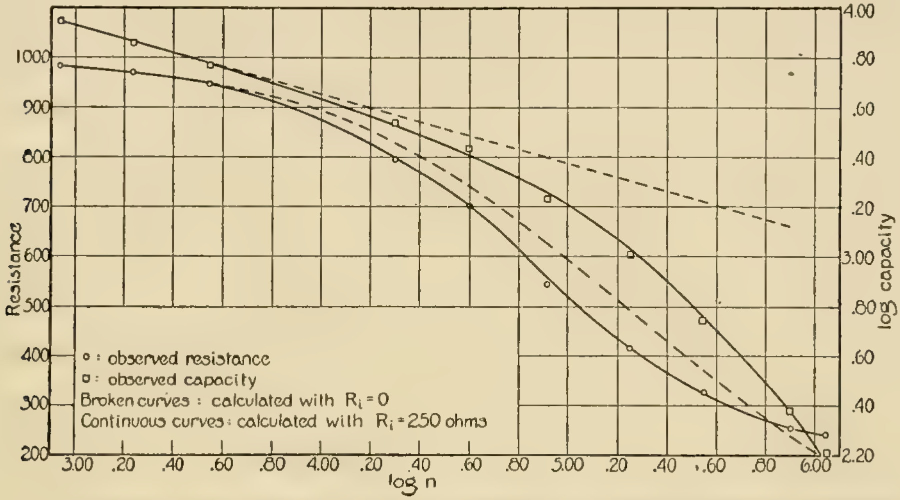

Fig. 5. Resistance and capacity of muscle of rabbit for currents of different frequencies.

The frequency dependance of C'_s is conveniently expressed by x, defined by the equation:

$$C'_s = C'_s\,(o)\,\omega^{-x} \qquad (15)$$

when x is constant, it can be shown theoretically[10] that m is also constant and related to x, by the following equation:

$$m = \tan. \frac{\pi}{2} . x \qquad (16)$$

The measurements of the suspension of spherical corpuscles, which are shown in Table 3, have been calculated by the equations stated. C'_s and $(R_i + R'_s)$ are derived from (12) and (13). x is calculated from (15) and is nearly constant*, equal to .035. The value $m = .06$ is derived from equation[16] and thereafter R'_s from [14]. R_i can now be calculated, and a test of the theory is that it remains constant.

For corpuscles of normal shape, equations (12) and (13) are only approximations. It is reserved for the future to extend them by consideration of the shape of the cells and the lack of homogeneity of the suspension.

The equations (7) to (16) have also been used in calculating data obtained for a variety of animal and vegetable tissues, and are often found to represent the experimental results with a considerable accuracy when a constant value of x is used. The same conclusion was arrived at by Cole[11], who has calculated measurements of a number of different investigators, using a graphical method, which, in substance, is identical with that used here.

***—Later more accurate measurements show x to be slightly larger at low, than at high, frequencies.**

Fig. 5 shows measurements of muscle[12] of a rabbit. The continuous curves are calculated with $x = .28$, $m = .46$, and $R_i = 250$ ohms. The agreement is very satisfactory. The dotted lines are calculated with $R_i = 0$, to show the influence of R_i.

Different tissues usually give nearly the same value of x, around .30, while m is around .50. The same values are also often found for the impedance observed at irresversible metal electrodes, like platinum in sulphuric acid[13] [14].

The value obtained for x for the red corpuscle is much smaller, being around .03. It is interesting to note that x is larger at low, than at high, frequencies. This is the opposite of what is usually found for other living cells, or for metal electrodes. But it is a type of variation which is very often found for the dielectric constant of solids, adding credence to the hypothesis[2] that the impedance of the red corpuscle surface is due to a non-conducting surface membrane, which acts as an electric condenser. On this theory m would represent the power factor of the membrane, (expressing the value of the dielectric loss).

Two different strains of bacteria (B. coli and B. mucosus capsulatus) tested[15], showed a behavior quite different from that of other cells which we have investigated. Their capacitance is very low and their conductance is nearly identical with that of the suspending medium, independent of its conductance. This is of importance in electrophoretic studies of bacteria. No proof was given though that any large percentage of the bacteria, used in these studies, were alive.

Table 6. Impedance measurements on rabbit corpuscles (30%) hemolized with water.

Kilocycles per sec.	R (ohms)	C ($\mu\mu$F)	C'_s (equation 12) ($\mu\mu$F)	x	$R_i + R'_s$ (equation 13) (ohms)
4	507.4	178.9		.043	
8	507.4	173.7		.025	
16	507.4	170.7		.018	
32	507.4	168.6		.022	
64	506.5	166.1			
128	505.0	161.5			
256	493.1	151.9	160.2		906
512	466.8	129.0	151.2		852
1024	414.4	91.9	143.3		810
2048	354.9	48.4	139.1		786
Solid saponin added	242	0			

A study[6]* of hemolysis of red corpuscles has given some interesting information. It is found that the hemolysis does not involve any change of the electric properties of the corpuscle surface, as far as may be ascertained by the present method. It might have been expected that the release of the hemoglobin would have left the surface with a high ionic permeability, but this is not the case. The surface retains its insulating properties and the value of the surface impedance is not changed. It is particularly significant, that the value of x is the same as for the normal corpuscle. This type of hemolysis is produced by amboceptor-compliment, by water (Table 6) and by saponin, when used in small concentrations. This conclusion is in agreement with results obtained by Abramson[16] by an electrophoretic study. Saponin, in large concentration, produces a complete destruction of the corpuscles. The hemolyzed solution is homogeneous as shown by the fact that the capacitance is zero and the resistance is independent of the frequency. Hemolysis by freezing and thawing is of a different type from that of the other lysms tested, involving a marked change of the surface impedance of the corpuscle.

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Discussion

Dr. Van Slyke: How did you determine the dielectric constant of the cell contents?

Dr. Fricke: The corpuscles were hemolyzed with solid saponin and measured in the bridge by substitution, comparing with a similar electrolytic cell filled with a salt solution of the same resistance as that of the corpuscles, and compensating for the difference in dielectric constants by a parallel condenser. Electrode polarization is the chief source of error to guard against.

Dr. Mudd: Can't the similarity in electrical properties between hemolyzed and unhemolyzed cells be explained on the assumption that the changes leading to the hemolysis occurred only at a few points on the cell surface?

Dr. Fricke: That is a possible explanation.

Dr. Ponder: That is my experience exactly, too. The lysis must take place locally in spots.

Dr. Abramson: These experiments of Dr. Fricke on hemolysis are in agreement with the results obtained by electrophoretic studies. I find that the electric mobility of red cells hemolyzed with water, saponin and immune serum (when the concentration is just enough to produce hemolysis) is, within 1 to 2%, exactly the same as the mobility of the normal cells, irrespective of the size and shape of these cells. This is confirmative evidence that the hemolysis take place in spots.

Dr. Mudd: Using more concentrated solutions of amboceptor it is possible to alter completely the wetting and electrophoretic properties of red corpuscles, and it seems probable that the electrical properties you have studied might also be altered in such circumstances.

Dr. Abramson: Dr. Mudd's statement is correct. All of us, who have been working on electrophoroesis of red cells hemolyzed with immune serum, find that the immune serum appreciably changes the red cell surface, when the concentration of the immune serum is comparatively high.

Dr. Fricke: That agrees with what we have observed. The addition of a large amount of saponin destroys the corpuscles completely, and the proof is that the capacitance is reduced to zero, and the resistance becomes independent of the frequency. The measurement of the impedance gives a means of determining whether or not any corpuscles, hemolyzed or not hemolyzed, are present.

Dr. Blinks: At how high a volume concentration of corpuscles is it possible to work?

Dr. Fricke: We use a standard clinical centrifuge, giving perhaps 4000 revolutions/min., and obtain volume concentrations up to 97%, as judged by the conductivity, on the basis of nonconducting corpuscles.

Dr. Blinks: What is the effect of very large currents on the corpuscles?

Dr. Fricke: We have noticed no influence of the current which could not be accounted for by the heat production.

Dr. Abramson: The increase in the specific resistance of the cell contents might be due to the fact that the hemoglobin is charged, and these charges produce interionic forces which diminish the conductance, in addition to the viscosity.

Dr. Fricke: If we assume that the hemoglobin molecules are spherical, and use Maxwell's formula for non-conducting particles, 30% hemoglobin would increase the resistance 60%. If the hemoglobin molecule is flat, the increase would be higher. There is, however, a marked difference in the equivalent conductances of KCl and NaCl, which is in a direction to give a lower interior resistance. This difference might partly be compensated by a small conductance of the hemoglobin molecule. However, in line with Dr. Abramson's remark, with respect to the relation of conductivity to osmotic pressure, we are on uncertain ground, when charged colloids are present. Consequently, to account accurately for the difference in the resistances is hardly possible at present. If we take account of the influence of the hemoglobin by means of Maxwell's formula, the effective viscosity, of course, is that of the conducting phase between the hemoglobin molecules. Another method of calculation would be to consider the corpuscle interior as a homogeneous medium, and take into account the lowering of the migration velocities of the ions, because of the high viscosity.

Dr. Blinks: Are you able from your results to draw any conclusion as to the conductance of the corpuscle membrane? McClendon considers this conductance to be rather high.

Dr. Fricke: Centrifuging corpuscles with care, a mass can be produced which has a specific conductance of about 1/3000 $ohms^{-1}$ (at room erature), but all, or a large part, of this conductance may be derived from the remaining serum.

The increase, at low frequencies, of the exponent x, may be related to the conductance of the membrane. As the frequency is lowered, the reactive component of the current decreases, and the conductance current would become more important. It is likely that the passage of the conductance current through the membrane would be accompanied by polarization, and this would give an increase of the capacitance, and therefore of the exponent x.

McClendon works with packed corpuscles centrifuged at speeds up to 20000 revol./min. The conductance of these packed corpuscles is often found to be very low. If, as he assumes, this high conductance is due to a high average conductance of all the corpuscles, we can only conclude that the corpuscles are abnormal, since much lower conductances can be obtained when we centrifuge with care. However, it appears more likely that the high conductance is due to the complete destruction of some of the corpuscles, while the others have retained their low conductance; because, if we calculate McClendon's data on this basis, the capacitance values for unit area of membrane, which are derived from his different experiments, agree quite satisfactorily, while his own calculation, based on conducting corpuscles, gives widely different values. However, even after this recalculation, his capacitance value is larger than ours. I think the reason is that electrode polarization was present as a serious error in his experiments.

Dr. Mudd: What test did you make for the viability of the bacterial suspension?

Dr. Fricke: We inoculated a drop of the bacterial suspension into sterile medium and obtained growth. Of course this would only prove that a few viable bacteria were present.

Dr. Mudd: Would the high conductance of the bacterial suspension mean that the surface membranes of the bacteria were freely permeable to ions?

Dr. Fricke: There is also the possibility that the capacitance of the surface of the bacteria was so large that, at the frequencies used, (1000 cycles/sec. and higher) the impedance of the surface was negligible.

Dr. Blinks: What was the concentration of your suspensions?

Dr. Fricke: 30% concentration.

Dr. Mudd: They would be in general, like dead plants.

Dr. Cohen: Since certain bacteria possess polysaccharide capsules of a thickness perhaps 10 to 20 times the diameter of the bacterial cell, it would follow that the mass of "inert" cellular matter would be very much greater than the "living," and therefore conductivity of such material would be essentially a measure of the predominating fraction (perhaps 1000 to 1) of the "inert" matter.

Dr. Riddle: Of course, to think of a living cell as taking up nearly the conductivity of a radically changed medium is quite contrary to what we will find for other living cells.

Dr. Fricke: Yes.

Dr. Mudd: Winslow and Falk did some work in which they speak of the buffering effect of bacteria on their environment.

Dr. Abramson: The measurement of the conductance of bacteria is of importance not only from the biological, but also from the physical, point of view, in connection with Henry's theory of electrophoresis.

Henry has recently obtained, by an analysis similar to that of Hückel, the effect of the specific conductance, λ', of a large particle migrating with a velocity, v, in an electric field of strength, X. He finds for a sphere, and subject to Smoluchowski's restrictions, that v is related to the specific conductance of the medium, λ,

$$v = \frac{3\lambda}{2\lambda + \lambda'} \, \frac{\zeta D X}{6\pi\eta}$$

($\zeta =$ electrokinetic potential; $D =$ dielectric constant of the medium, and $\eta =$ viscosity of the medium, both D and η remaining the same in the electric double layer).

Evidently if λ' is appreciable in the case of bacteria, ζ, and consequently the charge, will be influenced.

ELECTRIC CONDUCTANCE OF BIOLOGICAL SYSTEMS

Kenneth S. Cole

Since the time of Galvani, the electrical phenomena associated with the physiological processes of growth, stimulation, injury, recovery and death have received an enormous amount of experimental and theoretical attention. Certain of these phenomena, such as action potentials resulting from some physiological activity, and physiological activity, resulting from an externally applied electric field, may be considered as "active" from the physiological point of view. From the investigations of these activities, much evidence has been accumulated which indicates that in general the living cell may be considered to have an electrically conducting interior enclosed by a comparatively non-conducting membrane—or sheath. There is, furthermore, both direct and indirect evidence of the existence of an electric potential difference across this membrane in the normal resting cell. Also, almost any change of the physiological condition of the cell is accompanied by an alteration of this potential difference. Conversely, when the potential difference is changed by electric means there may be a change of the physiological state. It has become evident that the existence of this membrane potential is one of the most important criteria of the life of a cell. As a consequence, the factors which produce, maintain, and alter this membrane potential are of considerable interest. Since it seems quite certain that these factors are changed most markedly when there is any considerable change in physiological state, it is advisable to investigate those membrane phenomena by some means which involves as little physiological change as possible. In the so-called "passive" phenomena these considerable physiological changes and their accompanying processes of adaptation and recovery—which give added complications—should be largely eliminated.

We must treat the whole living cell—and in particular, the membrane—with the greatest care. It is advisable wherever possible to keep the cell intact, uninjured and resting. If we suppose the membrane potential to be of the order of fifty millivolts, it is to be hoped that there will be no great change in the state of affairs when this potential is increased or decreased a few microvolts, by the application of an external electric potential. When this small potential is applied we should expect some transport of net electric charge from the body of electrolyte on one side of the membrane to that on the other. This movement of charge will, of course, be due to movement of ions in the electric field. The number of ions moving, the rate at which they travel and how far they are displaced, will depend upon the nature of the opposing forces.

Membrane Forces

These forces may be divided into two groups, the dissipative, or non-conservative, and the storage, or conservative, forces. The former are primarily friction forces in which the energy is converted into heat, and in the case of cell membranes would be due to viscosity or friction phenomena of the medium in which the ion moves. In this case the ions will move with a uniform velocity as long as the field is present, and generate heat at a constant rate, but will stop in their tracks when the field is removed—very much as if they were being pulled by a string through molasses. A quite different state of affairs results from the presence of a conservative force acting on the ions. As examples of this type of force we shall consider those which involve the storage of potential energy (rather than kinetic energy)—such as the compression of a spring. Opposing forces of this type result when the flow of ions changes the osmotic pressure difference, the electric potential difference, or the ions move in molecular or elastic fields of force. In the ideal case, the ions would be entirely unopposed in the first instant of their motion, with a continual increase of the opposing force as a result of the ionic movement. Finally there may be built up opposing forces of such magnitude that there will be no further flow—so a counter e.m.f., is equal to the impressed e.m.f. If, then, the impressed e.m.f. is removed, the counter e.m.f. is still effective and tends to return the ions to their initial positions, thus releasing the energy which was stored during the first part of the cycle.

Electric Circuit Equivalents

In terms of purely electric network elements, the dissipative factors are represented by electric resistances in which the electric energy supplied is entirely converted into heat. The conservative factors are equivalent to an electric condenser consisting of a dielectric between two electrodes. The counter electromotive force is proportional to the charges placed on the electrodes and the energy used in charging the condenser is merely stored and may be regained outside. It should be noted that in this case there is no transport of ions across the membrane, and what would appear from a distance as a flow

through the membrane is merely an accumulation of charges of opposite signs on the two sides of the membrane. It may then be that an electric membrane resistance corresponds to a certain membrane permeability to ions, while a membrane capacity results from the accumulation of those ions whose passage through the membrane is opposed by conservative forces such as those mentioned.

If it were possible to measure directly the resistance and capacity of a variety of membranes the problem would not be so difficult as in the cases where we must deal with a tissue composed of many cells closely packed together, the membrane elements are found in all possible combinations with the elements representing the inter- and intra-cellular materials. If we are to make measurements of physiological significance, we should keep the tissue in as nearly normal condition as possible, and so about all that can be done is to place it between two electrodes. While we may then make any measurements that we wish at these two terminals—as long as the potential or current is kept sufficiently low—it must be remembered that there may well be more independent elements between these two terminals than there are independent measurements that we can make at the terminals.

Early Measurements of Conductance

The measurement of electric conductance is only one of the fields where discoveries and advances in the physical sciences have had an almost immediate use in living systems. Probably, however, no other biological application has followed the steps of physical progress quite so doggedly, or run into quite so many difficulties that were yet to be encountered in physical systems. Before 1800, it was known that an animal body conducted electricity, so it was only natural that immediate attempts were made at quantitative measurements when Ohm's Law was announced in 1827.

Direct Current

In contrast to physical systems, it was found for the human body not only that the resistance depended upon the magnitude of the current, the time that it had been flowing, and showed hysteresis, but that an e.m.f. persisted in the tissue after the source of current had been removed. The electrode troubles were serious enough in themselves, but the difficulties of interpretation were further increased when it was found that the resistance of skin under an electrode depended upon whether the electrode was anode or cathode. Nevertheless, there have been many investigations on the resistance of skin. The effects of salts, narcotics, temperature, pressure, injury and other pathological conditions have been measured. The resistances of other animal organs and various plants were also determined, but the following results are of particular interest. In general the resistance is lower at the make of the current than at any subsequent time. The resistance of live muscle is greater transverse to the fibers than parallel to them, while the resistance of dead muscle is lower and the same in all directions.

Alternating Current

The difficulties of measurements on the conductance of electrolytes were found to be practically as great as those on biological materials, until Kohlrausch substituted alternating current from the secondary of an induction coil in place of direct current as the source for a Wheatstone bridge. With this new method most of the electrode troubles were avoided. The resistances of biological materials remained constant as long as there was no physiological change, and were independent of the magnitude of the measuring currents when these were not too large. It was found that for alternating current the resistances were definitely lower than for direct current. Although the bridge could not be perfectly balanced by a resistance alone, a parallel condenser allowed a sharp minimum and precise values of resistance to be obtained. Since it has become possible to make precise and reproducible measurements, the electrical resistance of many tissues has been determined under a variety of conditions. Most of these investigations have been interpreted on the assumption that a major portion of the observed resistance was due to the cell membranes, and that changes in resistance were due primarily to changes in membrane permeability and resistance.

Resistance at Constant Frequency

The simplest experiments are those in which only the resistance is measured for a single frequency as a function of the physiological state. McClendon (1910) found that the resistance of centrifuged Echinoderm eggs decreased upon fertilization, and that the resistance of skeletal muscle decreased on stimulation (1912). The work on centrifuged marine eggs was repeated by Gray (1913, 1916) and confirmed (contra, Cole, 1928b). By far the most extensive and complete investigations of resistance at constant frequency (1000 cycles) are those of Osterhout (1912-22) on the kelp *Laminaria* and other marine plants. The resistance of normal *Laminaria* is about ten times that of sea water, while the dead tissue has a resistance about equal to sea water. Osterhout followed the time course of resistance changes in injury, recovery and death of the tissue resulting from various ions and narcotics, in particular. The changes in resistance of land plants accom-

panying stimulation and death have been investigated by Sen (1923), Ebbecke and Hecht (1923) Dixon (1924) and Luyet (1932). Crile, Hosmer and Rowland (1922) measured the resistances of various normal and pathological mammalian tissues, and particularly the changes in liver and brain resistance, resulting from fatigue, trauma, anesthetics, adrenaline and narcotics. Waterman (1922, 1923) not only showed that the resistance of malignant growths was less than normal tissue, but also that his factor which is proportional to the effective capacity of the cell membrane was increased. Changes in resistance of glands when stimulated have been found by Peserico (1926) and Bronk and Gesell (1926). Rapport and Ray (1926) recorded a decrease in the resistance of a turtle heart during isometric contraction. Shearer (1919) found salt effects on the resistance of bacterial suspensions similar to those for *Laminaria*, but the interpretations have been questioned, Green and Larson (1922), Zoond (1927).

Effect of Frequency

The observations that the direct current resistance was especially small at first and increased for some time after the current was started, and that an e. m. f. persisted after the external source was removed, suggested that there was some tissue element which had the characteristics of an electric condenser. The alternating current observations that a tissue could only be satisfactorily balanced when both resistance and capacity were used, and that the resistance was less than the constant direct current resistance, also suggest a condenser-like element.

The capacity C of an electric condenser is defined in terms of the charge q necessary to produce a potential difference e between its terminals, $e = q/C$. When a constant current i flows for a time t, $q = it$ and $e/i = t/C$. The apparent resistance, e/i, of a condenser to a constant current is then initially zero, but increases constantly. When the current is not constant, it is necessary to place

$$q = \int_0^t i\,dt \quad \text{so} \quad e = \frac{1}{C}\int_0^t i\,dt, \qquad (1).$$

When a sinusoidal alternating potential is applied to the condenser, $e = e_0 \sin \omega t$ *where* $\omega = 2\pi n$ and n is the frequency, it is found from (1) that

$$i = \frac{e_0}{1/C\omega} \cos \omega t.$$

The maximum value of the current "through" the condenser is a quarter of a cycle or an angle of 90° before the maximum potential difference. For a resistance, the two maxima are at the same time, so it is not correct to speak of the resistance of a condenser, and the term *reactance** X is used for the ratio of the maximum current,

$$X = e_0/i_0 = 1/C\omega \quad .$$

At low frequencies, ω is small, and the reactance is large, so very little current will flow through the condenser, while at high frequencies the reactance becomes small, so the condenser offers very slight obstruction to the current flow. When both resistances and capacity reactances are present, the situation is more complicated. The time between the current and potential maxima may be anywhere between zero and a quarter cycle, i. e. the *phase angle* is between 0° and 90°. The ratio of the maximum values of the potential and current in general is called the *impedance*. The impedance is a pure resistance when the phase angle is zero, and a pure reactance when the phase angle is 90°, and the performance of usual circuits may be given by the impedance and the phase angle. In order that two circuits be equivalent at a given frequency, it is necessary that both their impedances and their phase angles shall be equal. By varying the magnitudes of a resistance and capacity in series or in parallel, it is possible to obtain any impedance and phase angle, so these latter may be expressed either as a series resistance and reactance or a parallel resistance and reactance (Fig. 1).

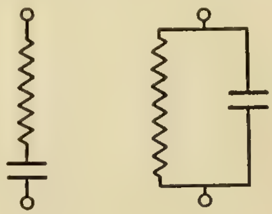

Fig. 1. Series and parallel, resistance-capacity circuits.

With three remarkable experiments, Höber (1910, 1912, 1913) gave the first demonstrations that the membrane of the red blood cell was the high resistance polarizable element postulated by Bernstein (1902). Such a membrane would have so high a reactance at low frequencies that practically all of the current would go around the cell —no matter how good a conductor the cell interior might be. This had been known experimentally for some time, but Höber showed that the resistance at high frequencies, where the reactance of the cell surface was negligible, was about the same whether the cells were intact or laked, and

* Since there is no experimental evidence or theoretical reason as yet to consider an inductive reactance in biological systems, the term reactance will be used to mean a capacitative reactance.

that the cell interior had a specific resistance between that of 0.1% and 0.4% KCl solution. With high frequency it was thus possible to hurdle the high resistance membrane and show that the cell interior was highly ionized.

Making use of the parallel condenser needed to balance the alternating current Wheatstone bridge accurately, Gildemeister (1919) found that as the frequency was increased, the series reactance and series resistance of human and frog skin steadily decreased until, at sufficiently high frequencies, the reactance vanished and the skin was equivalent to a pure resistance. If we let R and X be the series resistance and reactance and R_∞ the resistance for high frequency when $X = O$, we may consider the resistance $R\text{-}R_\infty$ and the reactance X as due to the cell membranes, and R_∞ as due to inter-, and intra-cellular electroyltes. For the membrane, the phase angle Φ is given by $\tan \Phi = X/(R\text{-}R_\infty)$. Gildemeister found this quantity approximately constant over the frequency range investigated.

Impedance Measurements

The first extensive use of the newly developed vacuum tubes for biological conductivity measurements was made by Philippson (1920, 1921). With a vacuum tube oscillator and voltmeter, and a substitution method, he measured the impedances of blood, muscle, liver and potato for frequencies from 500 cycles to 10 million cycles. In every case the impedance varied with the frequency in the manner shown in Fig. 2. Philippson interpreted this in terms of the circuit of Fig. 3a in which R_1 represents the resistance of the electrolytes inside and outside of the cells, R_2 and C the membrane resistance and capacity. In the first hour after removal, the value of R_2 for guinea pig muscle was about halved while R_1 re-

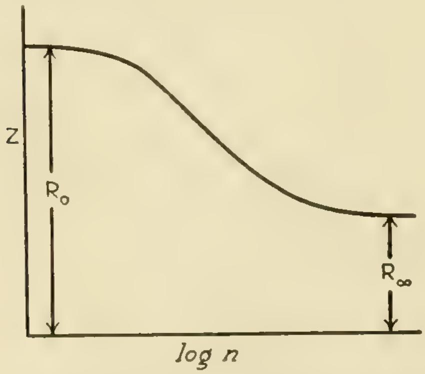

Fig. 2. Impedance Z vs. *log n* (n is frequency) for biological systems. R_0 and R_∞ are the limiting values of resistance extrapolated to zero and infinite frequency.

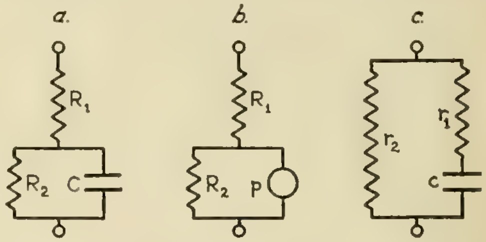

Fig. 3. Three circuits proposed as electrical equivalents of biological systems.

mained unchanged. For potato, the value of R_1 in germination was less than when resting, indicating an accumulation of electrolytes. Using a direct reading impedance method over a wide frequency range Cole (1928) obtained a value for the specific resistance of the interior of the *Arbacia* egg which was 3.5 times that of sea-water. Sapegno (1929) investigated impedance of frog muscle, and found R_2 measured parallel to the fibers was about half that measured in a transverse direction, while R_1 remained unchanged.

For the circuit of Fig. 3a it can be shown that

$$X = 1/C\omega = (R_o - R_\infty)\sqrt{\frac{Z^2 - R^2_\infty}{R^2_o - Z^2}}$$

where $R_o = R_1 + R_2$, the resistance at zero frequency, $R_\infty = R_1$, the resistance at infinite frequency, Z is the measured impedance of the tissue and X the membrane reactance at the frequency $n = \omega/2\pi$.

When the membrane reactance is computed by this formula, it is found in general that

$$X = X_o\,\omega^{-\alpha}$$

where X_o and α are constants. Philippson found $\alpha = 0.45$ for guinea pig liver and muscle, fresh and one hour after removal, and $\alpha = 0.25$ for potato, dormant and germinating. He did not publish the value of α for blood, but his data give 0.88. Cole found a value $\alpha = 0.5$ for the *Arbacia* egg, and Sapegno gives $\alpha = 0.37$ for frog muscle both perpendicular and parallel to the fibers. The reactance of a good condenser as given above is $X = 1/C\omega$ where C is the capacity independent of the frequency. If such condensers were present in the cells, we should find $\alpha = 1.0$, but the blood cell is the only case where $\alpha > 0.5$. Similar situations are found for metallic electrodes in electrolytes where this type of reactance has been interpreted as due to a condenser-like element having a capacity which depends upon frequency. These *polarization capacities* usually have associated with them a *polarization resistance* which also changes with the frequency. In

many cases, however, the ratio X_p/R_p remains constant over quite a frequency range, and since $\tan \Phi_p = X_p/R_p$ the phase angle Φ_p is constant (q. v. Fricke, 1932). When the condenser C of Fig. 3a. is replaced by a polarization element p, Fig. 3b, having the polarization resistance R_p and reactance X_p in series for instance, the formulation of Philippson has not been shown to be applicable. Unless $\alpha = 1$, it is probably safe to assume that polarization phenomena are present and it is to be expected that any value of $\alpha = 1$ is not correct.

Resistance and Reactance Measurements

As has been stated, a two terminal electric network is completely characterized at any one frequency either by the impedence and the phase angle or equally well by a resistance and a reactance. In general, the measured resistance and reactance are functions of the frequency, and these two functions $R(\omega)$ and $X(\omega)$ contain all of the electrical information to be obtained from alternating current bridge measurements. The interpretations of these functions must be based largely upon assumptions as to the electric and geometric structure of material between the two electrodes. It seems reasonable to assume that the resistance of the internal and external electrolytes is low compared to the thin but highly resistant cell membrane, and, as a first approximation, that all of the capacity is located in the membrane.

Single Cells

The most direct method of attack is to measure the resistance and reactance between the interior and exterior of a single cell, or between two points on the exterior such that most of the current traverses the cell membrane twice. This has been done with the large plant cells, *Valonia* and *Nitella* by Blinks with alternating current (1926) and direct current (1929 a, b). The alternating current data will be mentioned later. The direct current measurements lead to values of capacity between 0.1 and 1.0 μ F per cm.^{2}.

Suspensions

On the other hand, quite a bit is known of the current and potential distribution in and around the cells of a suspension which is not too concentrated. Considering the red blood cell as a spheroid, Fricke (1924) has derived a generalization of the Maxwell formula for spheres which predicts the resistance of a suspension of red cells extremely well. The membranes give a reactance component, and it is possible to calculate the capacity to be 0.8 μ F per cm^2 of the cell membrane (Fricke, 1925 a, b). Fricke and Morse (1925) then showed that a blood suspension could be represented over a wide frequency range by the circuit of Fig. 3c and found the corpuscle interior to have a resistance 3.5 times that of serum. On the other hand McClendon (1925) represented blood by the circuit of Fig. 3a. It should be emphasized that these circuits can be made identical in both impedance and phase angle for all frequencies, so the choice between the two is to be made on the basis of convenience of computation and interpretation. Intuitive interpretation may be misleading as can be shown by an extension of the Maxwell equation for spheres to include the reactance of a high resistance surface layer (Cole 1928a). It is then found that the specific resistances of the medium and the cell interior enter into the expressions for both R_1 and R_2 (Fig. 3a). In the other circuit (Fig. 3c) both specific resistances enter into r_1, but r_2 involves only that of the medium. Calculations of the specific capacity of the surface from the values of C (Fig. 3a) and c (Fig. 3c) are quite different and partially account for the discrepancy between values given by McClendon and Fricke for the red blood cell. At present, the resistance and reactance of suspensions of cells is the most powerful method available for the study of the electric characteristics of living cells, and it is unfortunate it has been applied to only the red blood cell in a satisfactory manner.

Tissues

In most tissues, the individual cells are so irregular in shape and the spaces between cells are so small that it has not been possible to formulate the gross tissue resistance and reactance in terms of the electric and geometric characteristics of the cells. Since the underlying assumptions become invalid for high cell concentrations, the analysis of suspensions should not be applicable to tissues, but may, nevertheless, be taken as a guide. The work on suspensions seems to justify the assumption that the cell membranes are the only elements having electric characteristics which depend upon frequency. When all the cells of a suspension are identical, it is found that the total effect of all the cell membranes is equivalent to a single element such as p of Fig. 3b having an impedance which is the same function of the frequency as the impedance of the individual cell membranes.* It is then to be expected that in tissues, the variable impedance element is situated at the cell membrane, and that for tissues composed of a single cell type, these membrane impedances are equivalent to a single variable impedance element. The circuit of Fig. 3b may then represent the tissue, since any resistance network containing a single

*—For the red blood cell this impedance is very nearly a pure reactance such as C of Fig. 3a but this is not generally true of tissues.

variable impedance element may be made equivalent to this circuit.

Interpretations of the resistances R_1, R_2 should be made with caution, but it is still possible to draw some conclusions as to the membrane characteristics from a consideration of this circuit and the measurements of the series resistance and reactance of the tissue $R(\omega)$ and $X(\omega)$ as functions of the frequency. Since the magnitude of the impedance $Z = \sqrt{R^2 + X^2}$ and its direction $\Phi = tan^{-1} X/R$, R and X may be considered as rectangular components of the impedance vector, so any pair of R and X at the same frequency determine the terminal point of the impedance vector as in Fig. 4. As the frequency is varied, this vector will change both in magnitude and direction, and its terminal will trace some form of curve. If the element p behaves like a condenser in that its impedance is very high at low frequencies, then no current will pass through p, $X(O)$ will be zero and the impedance will be a pure resistance, $R_o = R_1 + R_2$. At high frequencies, the impedance of p will be so low that there will be no potential drop across it, $X(\infty)$ will again be zero and the impedance will be $R_\infty = R_1$.

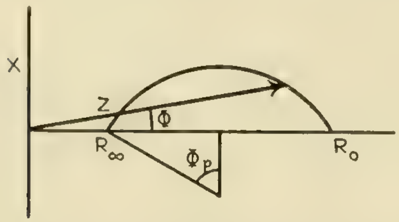

Fig. 4. Schematic circular arc locus of the impedance vector Z of an entire tissue having a single variable impedance element of constant phase angle.

The impedance locus will then have its end points at R_o and R_∞. In case p is a pure capacity, it is well known that the locus is a semi-circle of diameter $R_o - R_\infty$. Now let p be a polarization element consisting of a resistance R_p and a reactance X_p both of which change with frequency in such a way that their phase angle Φ_p remains constant or $\tan \Phi_p = X_p/R_p$. For this assumption it has been shown that the impedance locus is a circular arc—less than a semi-circle since the center is below the R axis—and that Φ_p is the half angle between the radii to R_o and R_∞ (Cole, 1928 a, 1932). There are several ways in which the value of the phase angle may be computed analytically, such as those used by Lullies (1930) and Fricke (1932). These may be preferable in cases where a good value of R_∞ can be obtained.

The experiments of Gildemeister on human skin and frog skin have already been mentioned to indicate their historical importance. With human skin R_∞ is approached at a relatively low frequency, and measurements have never been made at a low enough frequency to go beyond the maximum of the arc. The data of Gildemeister (1919) Einthoven and Bijtel (1923) and Hozawa (1925) give Φ_p in the neighborhood of 55° for human* and frog skin on the high frequency portion of the curve. Blinks (1926) found a similar frequency situation for *Valonia* with Φ_p about 55°. There are measurements on other tissues which fall in a convenient frequency range and may be used to test the hypothesis of a single constant phase angle element (Cole, 1932). It may be stated in general that, in the low and intermediate frequency ranges, the data are well represented by a circular arc, but that there may be deviations at the high frequency end. Fig. 5 shows data for potato which fit quite well, while the data of Lullies (1930) in Fig. 6 show the greatest divergence of any so far. The effect at high frequency is found in several tissues and may be due either to a failure of the polarization phase angle to remain constant at high frequency or to the presence of another impedance element which is masked by the high polarization impedance at low and intermediate frequencies. We may conclude that for at least the major portion

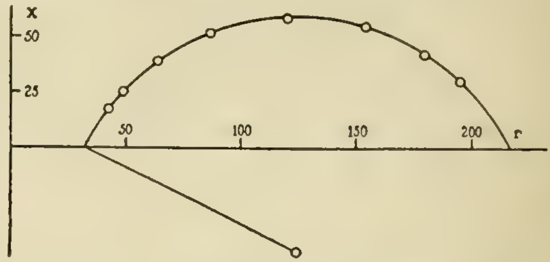

Fig. 5. Circular arc locus for potato. Resistance r and reactance x in ohms.

*—The recent data of Hozawa (1932 a) give a value of $\Phi_p = 89°$ for human skin.

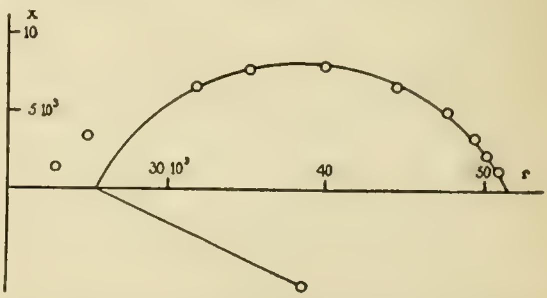

Fig. 6. Circular arc locus for nerve (Lullies). Resistance r and reactance x in ohms.

of the frequency range, the variable impedance element of living tissues—which is probably the cell membrane—has a constant phase angle. The constant value of Φ_p are given below for several tissues.

Tissue	Φ_p
Frog nerve (Lullies)	64°
Rabbit muscle (Fricke)	65°
Frog skin	55°
Human skin (above)	55°
Cat diaphragm	71°
Potato	64°
Laminaria	78°
Valonia (Blinks)	55°

Polarization

The classical polarization theories are those of Warburg (1899) and Nernst and Riesenfeld (1902) for reversible electrodes and diphasic systems. The former gives a phase angle of 45°. The latter gives a counter e.m.f. proportional to the square root of the time of constant current flow. This demands a 45° phase angle for alternating current. The reversible electrode may be considered the equivalent of a membrane which is perfectly permeable to one ion of a binary electrolyte. The alternating current extension of the diphasic system has been worked out in some detail by Hozawa (1931, 1932, b). From the data which are available, it seems that insofar as the phase angle of the polarization elements are constant, they lie between 45° and 90° for biological systems. The frequency variations of capacity and resistance found for irreversible electrode-electrolyte systems and dielectrics are examples of polarization in non-living systems. Usually when it is possible to make any generalization, the phase angle is approximately constant independent of frequency. There are, however, no satisfactory theoretical explanations.

Hozawa (1932 a) has interpreted his recent data on human skin in terms of a polarization of 45° phase angle and a static capacity in series. This accounts for the observed capacity very well, but does not give as satisfactory an explanation for the constant phase angle of nearly 90° observed above 1000 cycles.

Transient Conductance of Skin. Hozawa

The direct current flow in human skin at very short times after the application of a constant potential was measured accurately with a Helmholtz pendulum by Hozawa (1928 a). The assumption that there was no polarization—except that of a static capacity—explained the data very well as an exponential curve up to 50 micro-seconds*, and the progressive divergence after that time was attributed to diffusion polarization. These data then gave values of the capacity C and resistances R_1 R_2 of circuit Fig. 3a. $C = 0.02\ \mu$ F, $R_2 = 15{,}000$ to 116,000 ohms per cm². of skin area, and $R_1 =$ 400 ohms.

In the succeeding paper, Hozawa (1928 b) gave measurements of the damped oscillatory current when an inductance was included in the circuit and a constant potential applied. From the inductance and the observed frequency, the value of the capacity C could be computed. It agreed with the non-oscillatory value and was independent of the frequency in the range measured. This was extremely difficult to understand when compared with earlier alternating current bridge measurements where the capacity decreased with frequency and the phase angle was between 50° and 60°. The Fourier Integral analysis shows that an element which gives an exponential time relation for the current in a dissipative circuit with a constant potential applied, must give a capacity which is independent of the measuring frequency. Furthermore, an element having a constant phase angle, different from 90°, can not give an exponential direct current transient. The recent alternating current bridge data of Hozawa (1932 a) give a phase angle of almost 90° in agreement with the transient observations. This suggests that the earlier bridge measurements may have had some unrecognized source of error, and one is inclined to suspect that the measuring current may have been too large.

Conclusion

It was suggested at the beginning of this paper that electric conductance might be expected to furnish a clue to the mechanism of cell membrane phenomena. This it has so far failed to do. The alternating current conductance at low frequencies and the steady direct current conductance are found to be indices of the physiological condition of the cells. The high frequency alternating current conductance and the initial direct current conductance are comparatively independent of the physiological condition, and show that the cell interior has a comparatively high conductance. It may then be said that the cell membrane has a very low conductance which changes as the permeability of the membrane is altered. Although electric conductance is a useful empirical tool, it has not yet been possible to formulate it adequately in terms of membrane structure and mechanism. The experimental evidence at present indicates that the electrical be-

*—When one postulates a constant but unknown polarization in addition to a static capacity, a time interval of 100 micro-seconds may be accounted for.

haviors of cell membranes are very similar to those of the polarizations in solid dielectrics and at irreversible electrode surfaces—which are also unexplained.

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Discussion

Dr. Blinks: I might add to what Dr. Cole has reviewed of the work on tissues and cell suspensions, a few words on the same type of measurements with the large plant cells *Valonia* and *Nitella*. It was early recognized that such measurements should be made on these cells, and they proved to have certain advantages as well as disadvantages. The advantage lies chiefly in the large area with only two surfaces in series, so that the actual capacity is extremely large—up to 0.1 or 1.0 microfarad. This causes the complete change of impedance described by Dr. Cole to fall in a fairly low frequency range, usually below 20,000 cycles, which has the technical advantage of keeping the measurements largely in the audio range.

In general the same results have been found as Dr. Cole indicated for most plant and animal tissues except erythrocytes, namely, a decrease of the capacity itself with frequency, again less rapidly than the square root relation given by reversible electrodes, and a similar, though more rapid fall, of the associated series resistance, with increased frequency. This so resembles the phenomena at an indifferent electrode such as platinum in KCl, that we call it a polarization capacity and resistance.

But when we attempt to describe these time or frequency relations more accurately than by this rough characterization, we find a variety of difficulties, some peculiar to the organisms employed, others perhaps more widespread than is generally realized. One of these latter difficulties is implicit in the electrical circuit itself. The Maxwell formula for the relations of cell volume and surface to the outside solution seems to take care of this for suspensions: its best justification is the fact that Dr. Fricke finds a nearly constant capacity for the surface of erythrocytes. But for the closely packed, non-spherical cells of tissues, as well as for *Nitella* and *Valonia* where electrical contacts are made at the ends or other localized regions, we must consider also the capacity between the exterior and cell interior across the protoplasm *along* the cells, not perpendicular but par-

allel to the general axis of current flow. This may occur not only between the electrode-contact regions, but in some cases, as in nerve, and *Nitella*, under the contacts themselves. This is in essence the problem of "distributed" capacity, met with in telephone and other cables, where it has been mathematically solved by Kennelly. Both theoretically and experimentally such distributed capacity can give rise to frequency changes in the equivalent "lumped" capacity usually assumed for the circuit, in a manner so like those at a polarizing electrode that we must know pretty accurately the constants of the circuit before deciding which factor, polarization, or distribution, or both, are involved, and to what extent. I think this can be solved when we can keep some of the other things involved in the living protoplasm constant.

Here we run into those "active" effects of the electrical current, as distinguished from the "passive" ones we always hope to maintain during the measurement. In other words the current "does something" to the protoplasm when it passes across it. *Valonia* and *Nitella*, as normally employed in experiments, are in such condition that two opposite effects of current flow are beautifully exemplified in them. An impaled cell of *Valonia* has a small negative potential and polarizes only slightly to small currents, or even to very large currents passing outward across the protoplasm. But to increased currents passing inward, there is a striking response, the potential going through an S-shaped reversal curve, and reaching values of 200 mv. positive. It then polarizes quickly and regularly to increments or decrements of this current, and remains polarizable for a short, variable period after the current has ceased to flow. This seems to be a "restorative" effect of current flow, if we consider the cell in a normally "stimulated" state.

On the contrary, *Nitella* has normally a very high positive potential (up to 200 mv.) and polarizes promptly and regularly to small currents in either direction, or even to very large ones passing inward. But at the break of large inward currents, or at the make of rather small outward currents, the cell becomes stimulated, the potential drops to nearly zero, and the polarizability temporarily disappears. Alternating currents of sufficient intensity can also cause this stimulation, so we must work with very low currents.

During the past year methods have been found for "restoring" *Valonia* to polarizability, and maintaining it in that state, so that capacity measurements of impaled cells could be made, thereby avoiding the difficulties due to distributed capacity. Other difficulties due to the high resistance of the inserted capillary still exist, so that the problem is not solved. Osterhout and Hill have also found means for greatly decreasing the sensitivity of *Nitella* to electrical stimulation; I hope to employ such insensitive cells for capacity measurements.

One other disturbing point brought out by the direct current study of capacity is that the charging curve of the protoplasm has in many cases form, as well as speed, very different from the discharge. This would necessarily be also reflected in the A. C. measurements to some extent, and might give very strange frequency relations.

Dr. Cole: The possibility of distributed capacity and resistance must certainly be considered in all cases. These effects are, of course, present in the case of a suspended spherical cell, but they do not put in theoretical appearance in the unpleasant ways found for even the simplest cable problem. The impedance of suspended *Arbacia* eggs is then at least partial support for the belief that the membrane of itself has polarization characteristics. Fricke has found that the nearly constant capacity of red cells as found in suspensions is practically unchanged when the cells are centrifuged down to a cencentration quite comparable to that of cells in tissues. This may be taken as a further indication that the frequency characteristics of tissues are due more to polarization membranes than to distributed effects.

Nitella and nerve ,on the other hand, should be expected to show more of the characteristics of cables. It may be possible that these difficulties are somewhat avoided by suspending in air and making the electrode regions of short length. The *Nitella* cell wall is imbibed with tap water and not an especially good conductor so that most of the current will cross the membrane and travel through the cell interior. With short electrode regions the potential drop along the interior under the electrode would then be small compared to the potential drop across the protoplasm and distributed effects should be small. Nerve is more complicated because of the presence of intercellular electrolytes of high conductivity. Lullies data give a series of complex plane circular arcs for different lengths of nerve which are practically identical for lengths greater than 8 mm. except for a displacement along the resistance axis. This indicates that there is no current flow across membranes more than 4 mm. from an electrode region. An analysis similar to that of Rushton should give the current paths in the electrode region when the latter is long, and show what might be gained by the use of a short electrode region.

It should be pointed out that distributed capacity and resistance must of necessity enter in exactly the same manner whether alternating or direct current is used.

The difference of charge and discharge curves suggests a rather complicated mechanism, for, as

Kelvin said, "The charges come out of the glass in the inverse order in which they go in."

Dr. Fricke: I may mention some measurements on collodion membranes which I think may prove to be quite significant. A collodion membrane behaves very much like a cell membrane in that the current passing through it is strongly polarized. The polarization appears to be derived from the aqueous phase of the membrane, as evidenced by the changes produced when the membrane is placed in different salt solutions. In one case there may be a polarization of the type observed for the red corpuscle membrane with a very small frequency dependence of the capacitance. In another case, the polarization may be of the type found in tissues or at metal electrodes. At very high frequencies, the capacitance is independent of the salt solution and considered as the ordinary static capacitance of the membrane. This gives a dielectric constant of about 5, which is within the range of values found for the dielectric constant of cellulose.

Dr. Cole: Experiments with artificial membranes are extremely important. An attempt was made several years ago to find changes in conductance of frog skin as the selective permeability was reversed by altering the pH of the salt solution, but the measurements were not reproducible. More recently, a start was made on protein coated glass and collodion membranes for the same purpose, but the work has been delayed. The wax cuticle of the onion membrane has been found to give a constant phase angle, and is known to show a selective permeability. The non-aqueous liquid artificial membranes also need investigation.

Dr. Kornhauser: Do skeletal and smooth muscle show the same conductance phenomena? There has been so much disagreement on whether or not striated muscle really has true discs of Krause through the fibrils and sarcoplasm.

Dr. Cole: I do not know of any conductance data taken parallel and transverse to smooth muscle fibers. Sapegno's impedance data parallel to the fibers show polarization elements effectively normal to the current flow, but the frequency characteristics are the same as for the transverse measurements. This suggests that the fiber interior may be electrically isotropic and that the polarization observed is due to membranes which are not effectively parallel to the current flow.

Dr. Kornhauser: Then that would seem to indicate that there are no membranes across each minute distance between the middle of the isotropic bounds where Krause's membranes are located. Half way in the singly refractive band of each fibril there is a distinct disc shown in stained preparations.

Dr. Cole: I would think it probable that the discs are at least not electrically differentiated.

PHAGOCYTOSIS

Stuart Mudd

Phagocytosis* may be defined as the ingestion of a particle by a living cell. The particle may be non-living, or may be a bacterium or other cell, to whose death and digestion phagocytosis may or may not lead. In the present discussion, we shall be concerned merely with the act of ingestion. For historical treatment of phagocytosis and for discussion of many aspects not here dealt with reference is made to the classical monographs of Metchnikoff[1, 2] and to more recent reviews by Ernst[3], Marchand[4], Hamburger[5, 6], Neufeld[7], Neufeld and Loewenthal[8], Ledingham[9], Muir[10], Gray[11], Fleishmann[12] and Hirschfeld[13].

The phenomenon of phagocytosis has been traced by Metchnikoff through different groups of animals from protozoa to man. He pointed out that in amoeba and similar protozoa phagocytosis of bacteria and other organic material is the usual way of obtaining food. After ingestion the engulfed particles are either digested by cytoplasmic enzymes or extruded. In the lower metazoa, phagocytosis and intracellular digestion still play the principal rôle in the nutrition of the animal. Here cells, living in the body cavity, phagocytize and digest the food material. With greater specialization this primitive method of digestion gives way to the more complicated extracellular digestion in specialized hollow organs, such as the gastrointestinal tract. There remain in all metazoa, however, motile cells which have retained the primitive power of phagocytosis and intracellular digestion. In the higher vertebrates two principal categories of phagocytic cells may be distinguished, the polymorphonuclear leucocytes or *microphages,* and the *macrophages* or cells of the so-called reticulo-endothelial system[14]. But Lubarsch[15] and others[16] have stated that even in the higher vertebrates nearly all types of cells may, under some conditions, act as phagocytes.

Phagocytosis by the microphages and macrophages of vertebrates has been extensively studied both in vivo and in vitro. Various kinds of particles such as carmine, carbon, erythrocytes and other animal cells, and living or dead bacteria have been introduced by various routes (intravenously, subcutaneously, by injections into the serous cavities) into the intact animal. The fate of the injected particles has then been studied by making direct smears from exudates of organs, or by histological methods.

In vitro methods lend themselves to more quantitative treatment. Phagocytic cells, usually obtained from the blood or from exudates, are brought into contact with the test particles under various controlled experimental conditions. Smears are then made and stained, and the amount of phagocytosis determined either as the mean number of particles ingested per leucocyte, or as the percentage of leucocytes which have ingested particles under the given experimental conditions. Still another procedure was elaborated by Fenn [17]. In a phagocytic system with known numbers of cells and test-particles, Fenn determined the number of uningested particles as a function of time.

In this lecture attention will be confined to the two principal types of mammalian phagocytes, the polymorphonuclear leucocytes, and the macrophages or large mononuclear phagocytes. These cells are a principal factor in the interception and removal of any parasites or other foreign material which may reach the tissues or circulating fluids, and, also, in the removal of any débris resulting from tissue injury or tissue replacement.

In attempting to analyse the mechanism of phagocytosis, it will be convenient also to restrict attention to phagocytosis in vitro. Consider the process in two stages: first, the cell and particle come into contact; second, the cell ingests the particle.

The Probability of Contact Between Phagocyte and Particle

The first stage is best studied by simplifying the system as much as possible; this is done by suspending cells and particles in some liquid contained in tubes, which are slowly rotated. Under these circumstances it is evident that contact between cell and particle occurs purely by chance. Chemotropism is not involved since leucocytes are capable of locomotion only when attached to a solid surface; in other words they can crawl, but not swim.*

Under these simplified conditions it might be expected that the amount of phagocytosis would be proportional to the chances of collision between cell and particle, such was found by Fenn to be the case[17, 19, 20].

With a system consisting of rat leucocytes, quartz or carbon particles of known size and a suspending medium of isotonic NaCl solution and serum, he found that the rate of phagocytosis was

*** In preparing this lecture the writer has drawn upon an article for "Physiological Reviews" in preparation by Stuart Mudd, Morton McCutcheon and Balduin Lucké.**

*** Philipsborn finds some evidence to the contrary; see page 157 of his review on locomotion of leucocytes[18].**

proportional to the number of uningested particles. This relation is what one would expect at the beginning of an experiment, but that it would still hold after leucocytes had already ingested particles is interesting; it depends on a type of behavior previously found experimentally by McKendrick[21], i. e., that there is no decrease in the ease with which a cell engulfs one small particle after another. Observations of our own indicate that this principle is only true within limits; we have observed phagocytes so full of erythrocytes or Monilia cells that further phagocytosis was inhibited[22].

Fenn showed in two additional ways that phagocytosis is ordinarily proportional to the chances of collision. It is evident that the chances of collision depend on the size of particle and of cell—that is, according to Fenn's formulation, on the square of the sum of the diameters of cell and particle. The size of both cells and particles being known, it was possible to test this hypothesis by using particles of different size, and the relation was found to hold[17, 19].

Secondly, Fenn showed that the chances of collision depend on the relative velocities of cell and particle in the rotating tubes. These velocities can be increased or decreased at will by varying the speed of rotation; for when the tube is slowly rotated there is a relatively long time for the object to fall under the influence of gravity, before completion of rotation brings it back to its original position; whereas rapid rotation allows it less time to fall, so that it moves fewer millimeters per minute, until under very rapid rotation its rate of motion becomes practically zero. Evidently when both cell and particle have zero velocity there is the least chance for collision between them—theoretically no chance, while there is the greatest chance for collision during slow rotation. These predictions were confirmed experimentally[17].

Thus it was shown that the rate of phagocytosis is proportional to the chances of collision. But this is true, of course, only under constant conditions. Thus, change in the kind of test object greatly changes the rate of phagocytosis. Such a difference was shown by Fenn to exist between quartz and carbon: under certain conditions carbon was phagocytized 4 times as readily as quartz [20].

And this occurs even though the numbers of collisions be equal, as was demonstrated by Fenn with another method. In this, the suspension was run under a coverslip onto a slide, and observed under the microscope. In one such experiment in a certain microscopic field in 24 minutes, 36 contacts between leucocytes and quartz particles were observed and 37 between leucocytes and carbon particles; yet at the end of the period only 1 quartz particle had been ingested as compared with 12 carbon particles[23]. Serum was present in Fenn's phagocytic mixtures, and Fenn states that little phagocytosis of either carbon or quartz occurred without serum. The differences between carbon and quartz are probably referable, therefore, chiefly to differences in the ability of carbon and quartz to adsorb phagocytosis-promoting substances from the serum under the given experimental conditions.

With this method, called by Fenn the "film" method, contact between cell and particle results not from motion induced by gravity, as in the preceding experiments, but from amoeboid motion, the cells crawling about, coming in contact with particles which they then may or may not ingest. Under these conditions a complicating factor may be introduced, chemical attraction of particle for cell. This was not the case with particles of quartz and carbon; with these, contacts appeared to be purely by chance. When, however, particles of MnO_2 and of $MnSiO_3$ were used, it was evident by direct observation that the collisions were no longer fortuitous. Though equal numbers of the two kinds of particles were present, 2.4 times as many encounters occurred with MnO_2 as with $MnSiO_3$; this resulted in 20 times as many MnO_2 particles being ingested as $MnSiO_3$. There was evidently definite chemical attraction exerted by the MnO_2 particles, a conclusion which Fenn confirmed by observing that leucocytes frequently advanced directly toward these particles instead of exhibiting the usual random movements. The chemical attraction he supposed to depend on the fact that MnO_2 is soluble in water, though only slightly so[23]. Similarly Commandon[24] was able to demonstrate by moving pictures that leucocytes are attracted toward starch grains which are subsequently ingested.

Electrokinetic Potential and Surface Charge

An obvious question in considering the approach and contact of leucocyte and particle in vitro is that of the effect of their electrokinetic potentials.* It is not to be anticipated that ζ-potentials would have the same critical importance in phagocytosis as, for instance, in phenomena of aggregation of hydrophobic colloids. One reason for this is that the opportunities for collision of colloidal particles are ordinarily afforded by forces of a low order, namely Brownian motion, whereas the contact of leucocyte and particle in experiments in vitro are afforded either by mechanical agitation, or by the locomotion of the leucocytes. Another reason is that the ζ-potentials of leucocytes are relatively low; Abramson

* Discussion of these factors in vivo has been given by Abramson in a previous lecture of this series.

gives for horse polymorphonuclear leucocytes in serum the value of approximately 12.5 millivolts [25].

The attempt to determine from experimental data the effect of ζ-potential on phagocytosis is rendered difficult by the fact that the conditions which have modified the ζ-potentials have often simultaneously modified also the interfacial free energies, upon which phagocytosis is more directly dependent. Cases in point are the promotion of phagocytosis by treatment of bacteria with tannin[26], or with serum[27]. Moreover, influences which have been attributed to the direct effects of ions on phagocyte or particle have often not taken into account the effects of such ions on the adsorption of serum proteins on the bacteria.

One interesting set of experiments in which such disturbing effects seem to have been reduced to a minimum, is that of Neufeld and Etinger-Tulczynska[28]. In these, leucocytes washed and resuspended in isotonic NaCl solution were used. The bacteria were grown on solid media, and were washed and resuspended in distilled water. To these suspensions dilute electrolyte solutions were added. Solutions of salts of polyvalent cations in certain ranges of concentrations both agglutinated and caused phagocytosis of a strain of virulent pneumococci and of a typhoid bacillus. The minimal concentrations able to bring about agglutination and phagocytosis were approximately the following:

> $Th(SO_4)_2$, N/2000; "aluminum alum," 1:5000; $Al_2(SO_4)_3 \cdot 18H_2O$, N/2000; $AlCl_3 \cdot 6H_2O$, N/1000; $Al_2(NO_3)_6 \cdot 18H_2O$, N/2000; $Fe_2(SO_4)_3 \cdot 9H_2O$, N/500; $Cr_2(SO_4)_3$, N/500; $CeCl_3$, N/1000; $Pb(NO_3)_2$, N/2000.

Di-divalent salts were not found to be effective. Although ζ-potentials were not determined, these experiments were interpreted as being due to reduction of electrokinetic potentials by these salts.

Numerous parallel determinations of ζ-potential and phagocytosis have been made by Mudd, Lucké, McCutcheon and Strumia. A number of acid-fast bacteria[29, 30], and collodion particles with proteins adsorbed on them[31], were examined in 0.85% NaCl solution, and also after treatment with serial dilutions of specific immune sera. It was found that in general the highest ζ-potential and the least phagocytosis occurred in the pure NaCl solution, and conversely the lowest ζ-potential and greatest phagocytosis occurred after treatment with the highest concentrations of serum.

The opposite relation between ζ-potential and phagocytosis is illustrated in experiments with typhoid bacilli[32]. These bacteria (in their "smooth" form) have only a minimal ζ-potential[33]; in the presence of homologous immune serum they acquire a negative ζ-potential. Yet sensitization greatly promotes phagocytosis. These correlations, both direct and inverse, between ζ-potential and phagocytosis we believe to be accidental and dependent upon the fact that sensitization involves the deposition of a film of antibody-globulin on the bacterial surface.

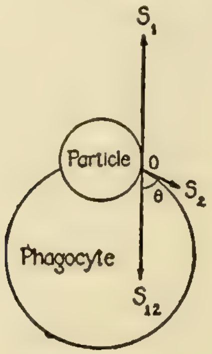
That ζ-potential is not the decisive factor in determining phagocytosis appears also when we consider different acid-fast bacteria in NaCl solution, and attempt to correlate their ζ-potential and ingestion. No correlation is found. Thus in absence of serum, virulent human mammalian tubercle baccilli were freely phagocytized although they have a relatively high ζ-potential, while, conversely, M. avium (Prague strain) with a relatively low ζ-potential was not spontaneously phagocytized[27, 29]. Other bacteria might be selected to show the opposite relation.

The subject of the influence of surface *charge* (not to be confused with ζ-potential of which it is a complex function) on the phagocytosis of a particle has been treated by Ponder[34], who has elaborated Gyemandt's equations, for the effect of surface tension and charge on the contact of two like particles, to cover the case of the ingestion of a particle by a cell. The results are exceedingly complicated, and the equations are experimentally unverifiable, but three interesting conclusions are arrived at:

> (1) Only large surface charges at the cell-fluid and particle-fluid interfaces can influence the ingestion. Very large charges at these interfaces will prevent ingestion.
>
> (2) If a very large charge occurs at the cell-particle interface ingestion may be prevented.
>
> (3) The effects on ingestion of the charges at these three interfaces vary with the radius of the cell, the radius of the particle, and the surface tension at the interfaces, in such a complex way that the effects of surface charge on phagocytosis is probably comparatively small.

Interfacial Tension Relations

It was first shown by Fenn[35] that the same formulation of the interplay of surface forces in phagocytosis may be reached from considerations of surface tension or of free surface energy. We shall use the former as simpler. Let text-figure 1 be a section through suspending medium, phagocyte and partially ingested particle. Let O be a representative point in the line of contact between the three phases; let the vectors S_1, S_2 and S_{12} be the interfacial tensions, respectively, in the particle-fluid, phagocyte-fluid, and phagocyte-particle interfaces.



Text-Fig. 1

If $S_1 > S_{12} + S_2$ the surface of the phagocyte would be drawn completely around the particle and ingestion would occur, provided viscosity, or other forces, did not interfere with the action of the surface forces. If $S_{12} > S_1 + S_2$ neither ingestion nor adhesion of particle and phagocyte would occur under the action of surface forces. When $S_1 < S_{12} + S_2$ and $S_{12} < S_1 + S_2$, the surface forces are in equilibrium with the particle in a position of partial ingestion, as shown in the figure; the position taken by the particle at equilibrium is such that $S_1 = S_{12} + S_2 . \cos \theta$, or

$$\cos \theta = \frac{S_1 - S_{12}}{S_2} .$$

The three main possibilities have been expressed by Ponder in terms of the value of $(S_1 - S_{12})/S_2$ as follows:

$(S_1 - S_{12})/S_2$ is less than or equal to (-1). The cells will not ingest the particle under these circumstances, for the particle will either stay in equilibrium at the cell surface, or, if its physical nature permits, will flow over the cell.

$(S_1 - S_{12})/S_2$ is greater than (-1) and less than $(+1)$. In this case there will be a real value of θ, and there will be equilibrium at incomplete ingestion, i. e., when the particle is only partly inside the cell.

$(S_1 - S_{12})/S_2$ is equal to or greater than $(+1)$. In this case, given sufficient time, the cell will completely ingest the particle.

It follows immediately from these considerations that the probability of phagocytosis is favored by increase in the value of the particle-suspending medium interfacial tension (S_1), and by decrease in the particle-phagocyte interfacial tension (S_{12}). Phagocytosis in the body is promoted by the deposition on the particle surface of specific and non-specific serum proteins, and such serum sensitization is also the most reliable and powerful means yet discovered of promoting phagocytosis in vitro. The action of sensitizing serum in forming on bacteria a surface deposit of serum-globulin is to give them surfaces presumably with high interfacial tension against the medium (as evidenced by the hydrophobic aggregation behavior of sensitized bacteria),[33] and presumably with low interfacial tension against the leucocyte.

Unfortunately none of the three interfacial tensions in the phagocyte-particle-liquid system can be directly measured. We are forced to make such inferences as we can from analogy, and from indirect evidence.

In the case of the eggs of certain marine invertebrates K. S. Cole[37] and Harvey[38] have been able by independent methods to assign an upper limit to the protoplasm-suspending medium interfacial tension. The maximum values for the tensions at the surface of eggs studied by Harvey by the microscope-centrifuge method were:

Unfertilized egg of *Arbacia punctulata,* 0.2 dynes per cm.

Unfertilized egg of the annelid *Chaeptopterus,* 0.33 dynes per cm.

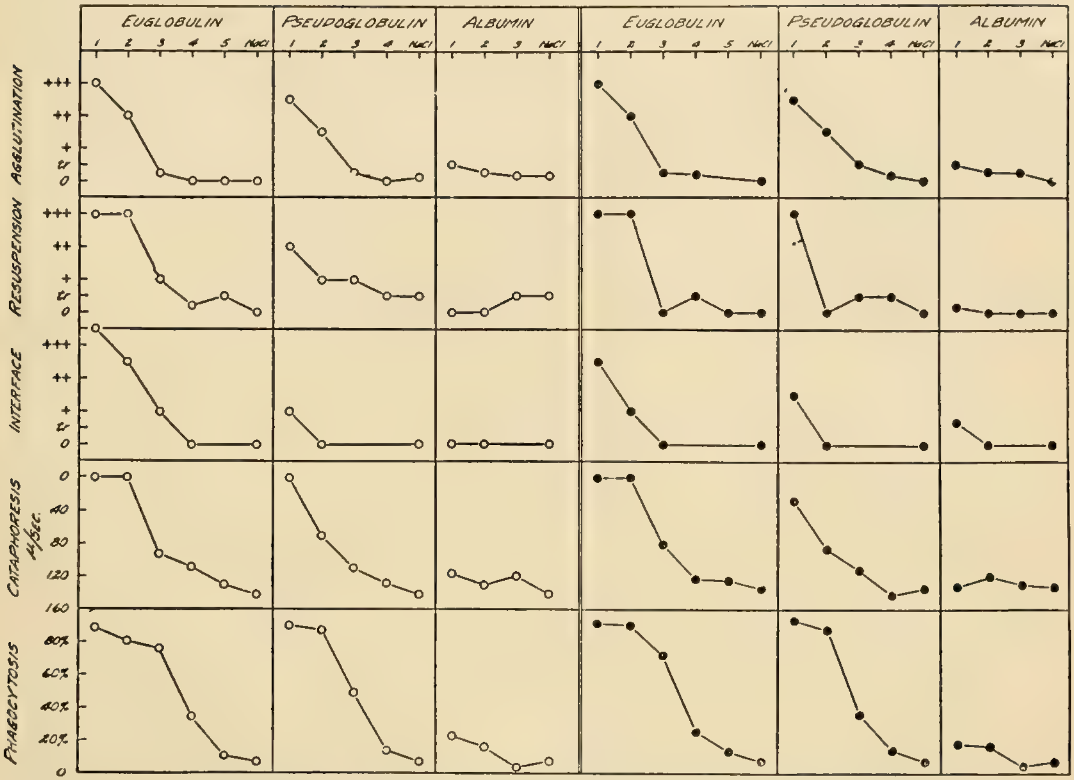
Egg of the mollusk *Cumingia,* 0.54 dynes per cm.

Fertilized egg of the mollusk *Illyanassa,* 1.1 dynes per cm.

Dr. Cole by computation from observations of unfertilized *Arbacia* eggs under compression, reached a value of 0.08 dynes per cm.—a remarkable agreement with the result of Harvey. He concluded: "It thus seems reasonable to suppose that there are no capillary forces acting, and that the initial pressure and tension are due to an initial stretch of this elastic membrane."

To regard such results as affording more than a suggestive analogy with the phagocyte-plasma interface would, of course, be unwarranted. There are however, independent reasons for believing that the tension at the phagocyte-liquid interface is low. In the blood stream leucocytes are typically spheroidal. However direct observation of these cells, when spread out on a surface, shows that their surfaces are highly mobile; the mononuclear phagocytes often project and retract into the medium in response to internal stimuli, delicate veil-like pseudopods; the polymorphonuclear leucocytes in their locomotion often leave long thread-like processes attached to the surface on which they are crawling[39]; these may pull loose and be retracted into the cell. The even more elaborate development of these "pseudopodes pétaloids" in the amoebocytes of invertebrates has been described by Fauré-Fremiet[40].

It is interesting to compute the amount of work involved in the extension and retraction of such processes. The phagocytes of mammals and the amoebocytes of invertebrates can both pass re-



Text-Fig. 2. Parallelism between surface changes and phagocytosis after sensitization with globulin fractions of immune serum. **M. avium** (Arloing strain) sensitized with serum fractions and then washed. Open circles: fractions from Anti-Arloing serum heated for 30 minutes at 56°C. before fractionation. Black circles: fractions from same Anti-Arloing serum unheated. Ordinates are the intensities of the several reactions. Abscissae are successive dilutions of antiserum fractions expressed in powers of 4. (Thus 3 is a dilution of $1{:}4^3$ or 1:64.)

versibly from the spheroidal form to the form in which surface processes are extended. According to the computations of Fauré-Fremiet, the amoebocytes of *Asterias* equivalent to 1 gram dry weight, in the condition of maximum extension have an aggregate surface area of 21.41 sq. meters; their surface in the form of minimal area has been computed as 3.03 sq. meters. Let us assume that some metabolic process provides to these extended processes sufficient surface energy to cause their retraction into the cell. The loss of surface area is then 21.41 — 3.03 = 18.38 sq. meters = 1.838×10^5 sq. cm. The work (W) required to increase the energy at the protoplasm-liquid interface by 1 erg per cm.^{2} is thus 1.838×10^5 ergs.

$$1 \text{ erg} = \frac{1}{4.18} \times 10^{-7} \text{ gram-calories.}$$

$$W = \frac{1.838}{4.18} \times \frac{10^5}{10^7} = 4.4 \times 10^{-3}$$

calories per erg/cm^2. per gram dry weight of amoebocytes.

Warburg[41] gives the energy liberated by surviving carcinoma tissue as 0.04 calories per milligram dry weight per hour. Fleishmann and Kubowitz, using Warburg's technique[12] obtained for rabbit exudative leucocytes 0.021 calories per milligram dry weight per hour. The latter figure is equivalent to 21/60 = .35 calories per gram per minute.

Were this energy transformed wholly into free surface energy it would thus be sufficient to increase in one minute the surface energy over an area equivalent to that of the maximally extended amoebocyte cells by $\frac{.35}{4.4} \times 10^3 = 80$ ergs per sq. centimeter.

It is thus clear that the energy made available in metabolism is far in excess of that required in the reversible extension of protoplasmic surface processes.

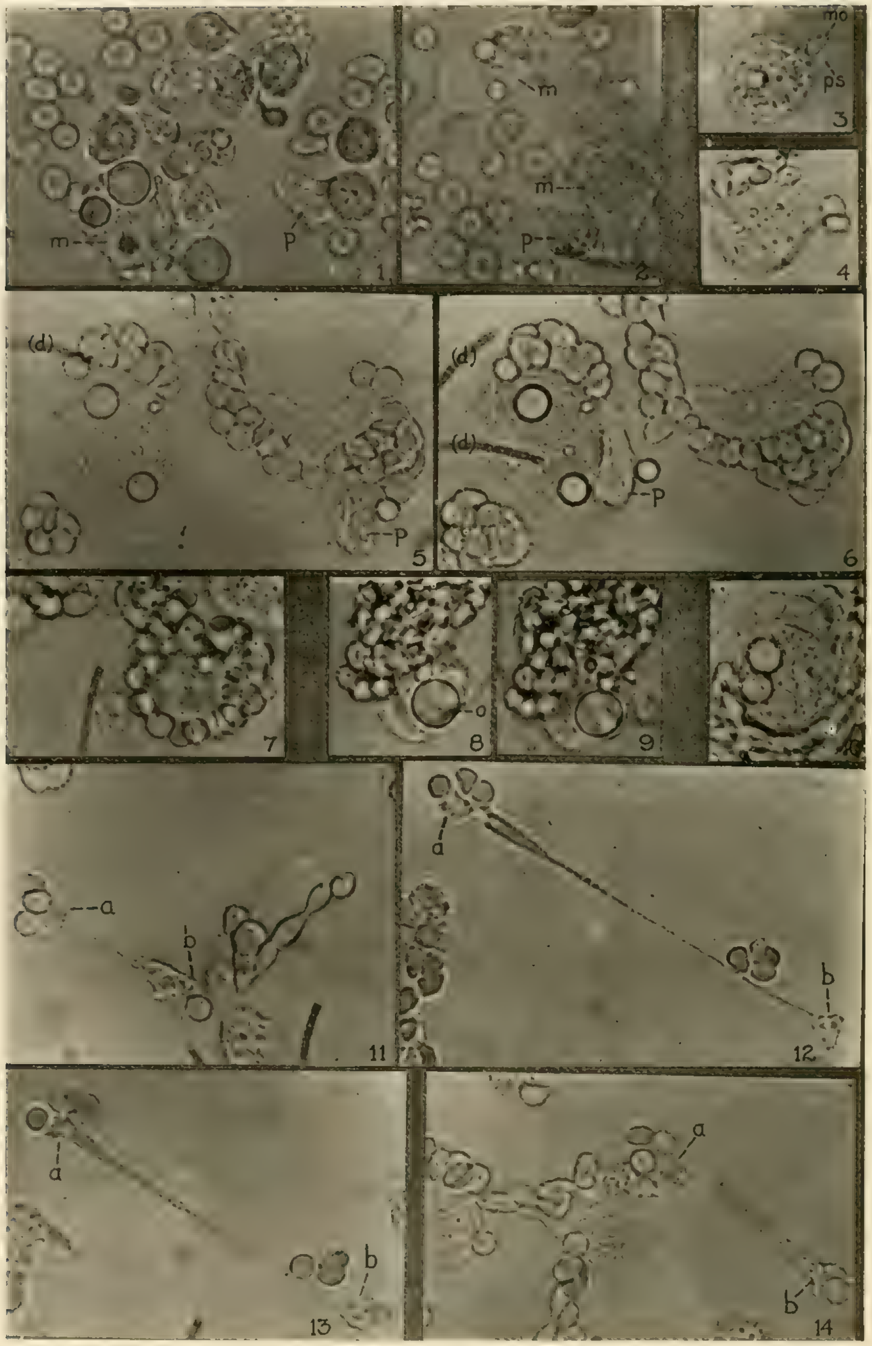


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PLATE 1

Experimental Analysis and Direct Observation of Phagocytosis

It remains to consider how far the formulation of phagocytosis as primarily determined by interfacial tension relations at the particle-cell-liquid interfaces has been verified by experimental analysis and by direct observation. Three deductions may be examined. These are (1) that a quantitative correlation should exist between phagocytosis and the surface properties of the particles ingested; (2) that phagocytosis is essentially a phenomenon of spreading of the phagocyte surface over the surface ingested; and (3) that partial ingestion should occur under certain circumstances.

Correlation Between Phagocytosis and Surface Properties. The first obvious deduction from a theory which assigns to surface forces a principal part in phagocytosis is that phagocytosis should be related, in some orderly way, with the surface properties of the particles phagocytized. This relation has been verified over a very considerable range of experimental conditions, [27, 29, 30, 31]. Various bacteria, erythrcytes, and protein adsorbed on collodion particles have been treated with graded concentrations of the phagocytosis-promoting substances of sera. The ζ-potentials, isoelectric points, wetting properties, and cohesiveness of such series of sensitized particles have been estimated in independent tests, and the phagocytosis of the particles has been quantitatively determined, using mammalian phagocytes both of the polymorphonuclear and large mononuclear types. [31, 42]. A remarkably close correlation between phagocytosis and the surface properties of the particles undergoing ingestion has regularly been found. As the surface properties of the test particles were altered step by step in the series of serum dilutions, phagocytosis was increased in close parallelism. (An example is given in Text-figure 2). The conclusion drawn from this work is that the phagocytosis-promoting substances of immune sera form, on the particles with which they interact, a surface deposit upon which phagocytes can spread [31].

We shall compare also the other deductions from the physical theory with the behavior of phagocytes under direct observation.

Methods of Obtaining Phagocytes. Exudative polymorphonuclear leucocytes [27] and large mononuclear phagocytes (macrophages) [42] have been obtained from the peritoneal cavity of rabbits by methods elsewhere described. These were washed in 0.85 per cent NaCl or Ringer's solution, and suspended in slightly diluted rabbit serum. In the major part of the work the cells used were samples from the same lots used in quantitative phagocytosis experiments [32, 42]. Human polymorphonuclear leucocytes were used in a number of experiments. A platinum loopful of leucocyte suspension, a loopful of the suspension of particles to be phagocytized, and a loopful of specific immune rabbit serum were placed on a carefully cleaned slide, mixed, and a clean cover-slip was gently lowered on top. The edges of the cover-slip were sealed with Salvoline. In such

Plate 1

All figures are unretouched photographs of living phagocytes.

Figs. 1 and 2. Macrophages (*m*), polymorphonuclear leucocytes (*p*), and sheep erythrocytes. The large macrophage in Fig. 1 contains three ingested oil droplets. No sensitizing immune serum present; little or no agglutination or phagocytosis of erythrocytes.

Fig. 3. A macrophage ingesting a sensitized cell of *Monilia albicans* (*mo*). Note process of macrophage (*ps*) spreading around monilia.

Fig. 4. A macrophage ingesting sensitized sheep erythrocytes. At top erythrocytes being drawn into macrophage. On right a process spreading over two erythrocytes.

Figs. 5 and 6. Partial ingestion of weakly sensitized sheep erythrocytes by macrophages and polymorphonuclear leucocytes (*p*). The shadows marked (*d*) are dust on the camera lens.

Fig. 7. A cluster of strongly sensitized erythrocytes surrounding and being drawn into macrophage.

Figs. 8 and 9. Successive stages in ingestion of a mass of strongly sensitized sheep erythrocytes by a macrophage; the macrophage contains an oil droplet (*o*).

Fig. 10. A macrophage embedded in and ingesting strongly sensitized sheep erythrocytes. The macrophage contains two previously ingested oil droplets.

Figs. 11-14. Successive stages in migration of polymorphonuclear leucocyte which has partially ingested weakly sensitized sheep erythrocytes. That portion (*a*) of the leucocyte to the left of the figure has partially ingested three erythrocytes which remain adherent to the glass slide; the portion (*b*) of the leucocyte which contains one partially ingested erythrocyte continues to migrate toward the lower right hand corner of the field until the protoplasm of the leucocytes is stretched into a thin filament. While under direct observation the adherent erythrocytes in the upper left hand corner were pulled loose from the glass and the protoplasmic filament contracted.

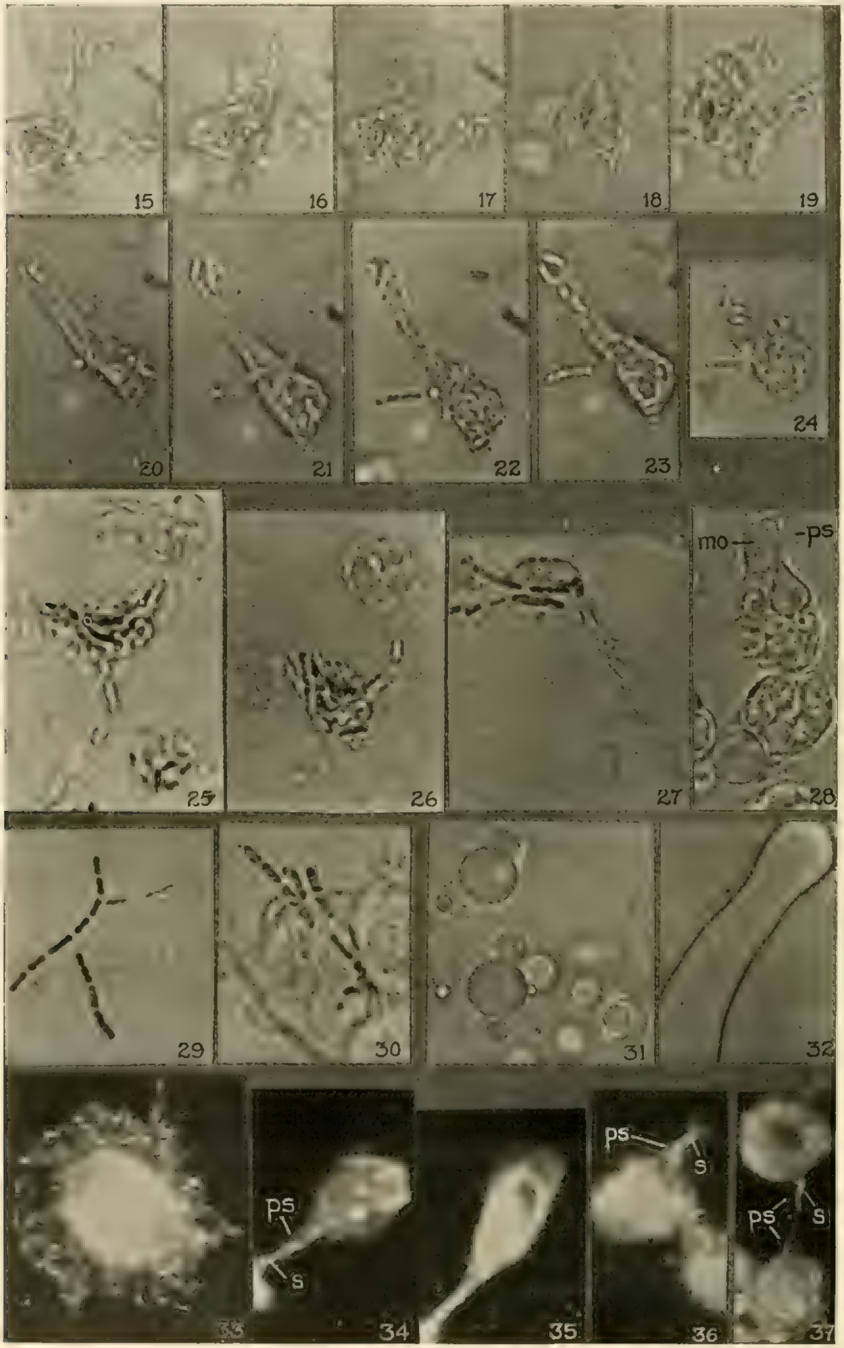

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PLATE 2

films some leucocytes were freely suspended, some were spread out on the slide, and some on the cover-slip; only rarely was a single cell in contact both with slide and cover-slip. The preparations were put immediately under microscopic observation in a warm-box kept near 37°C.

Particles Phagocytized. Suspensions of washed sheep and washed chicken erythrocytes, *Bacterium typhosum, Bacillus subtilis,* and *Monilia albicans* were used. Specific rabbit antisera were prepared for the sensitization of each type of cell. For observation and photography of the bacteria with the brightfield, they were first stained with carbolfuchsin and then washed four to five times. Erythrocytes and monilia were not stained. With the dark-field no staining was necessary.

Optical Apparatus. For transmitted light, Zeiss aplanatic N. A. 1.4 condenser. For dark-field, Zeiss cardioid condenser. Zeiss apochromatic 60 x objective with iris diaphragm. Zeiss 20 x compensating ocular. Zeiss microscope incandescent lamp No. 1 with 165 watt Mazda projection bulb. Zeiss Phoku camera. Hypersensitive panchromatic plates. For transmitted light, yellow G filter No. 15. Exposure time with transmitted light, 3 seconds; with dark-field, 30-60 seconds. Developer D 11 contrast (Eastman). Developed 5 minutes, room temperature. The superficial protoplasm of the phagocyte and the multiform processes and membranes to which it gives rise can be seen more clearly with the cardioid condenser than with any other optical arrangement with which we are familiar.

Phagocytosis a Phenomenon of Spreading. It follows both from the mathematical formulation of Fenn [35] and Ponder [36], and from the experimental analysis, that the capacity of the phagocyte to spread over the surface of the particle undergoing ingestion is a principal factor in determining phagocytosis. Is this deduction in agreement with the process of phagocytosis as directly observed? This question we have examined with especial care. Prediction and observation have been found to be uniformly in agreement.

Plate 2

Figs. 15-19. Successive stages in ingestion of clumps of strong sensitized typhoid bacilli by a polymorphonuclear leucocyte. In Figs. 15, 16, and 17 a process of the leucocyte spread over a clump of sensitized bacteria shown above the leucocyte. In Figs. 18 and 19 this process contracted, drawing the ingested bacteria toward the center of the cell. In Fig. 19 a second process began to spread over a clump of bacteria to the right of the leucocyte.

Figs. 20-24. Successive stages in contraction of the process of a polymorphonuclear leucocyte which has spread over a clump of sensitive typhoid bacilli. In Fig. 24 the process has contracted and the bacteria have moved in toward the center of the cell.

Figs. 25 and 26. A polymorphonuclear leucocyte spread over agglutinated *B. subtilis.* In Fig. 26 the cell is tending to round up and some bacteria have moved toward the center.

Fig. 27. A polymorphonuclear leucoctye spread over a λ-shaped chain of *subtilis* bacilli.

Fig. 28. Two polymorphonuclear leucocytes each filled with monilia cells. A process (*ps*) of the upper leucocyte has just spread around a monilia cell, (*mo*), and the lower leucoctye is spreading over another half-ingested monilia cell.

Fig. 29. A macrophage ingesting *subtilis* bacilli. Two chains of bacilli are adherent to the macrophage surface; the right hand arm of the upper Y-shaped chain has been drawn into the macrophage.

Fig. 30. *Subtilis* bacilli being drawn into a macrophage.

Fig. 31. Macrophages ingesting or spreading on the droplets of an emulsion of mineral oil.

Fig. 32. Macrophages spread out on a peninsula of mineral oil.

Figs. 33-37. Are dark-field photographs.

Fig. 33. Macrophage spread out on glass. In order to bring out the detail of the peripheral hyaline protoplasm, the detail of the central granular protoplasm has been lost by overexposure.

Fig. 34. Macrophage extended to a pear-shape by spreading over a *subtilis* chain. The *subtilis* chain (*s*) is the stem of the pear and the vague white around it (*ps*) a process of the macrophage.

Fig. 35. The same macrophage a few minutes later. The cell has thrown out thin "veil-like processes" toward the top of the picture.

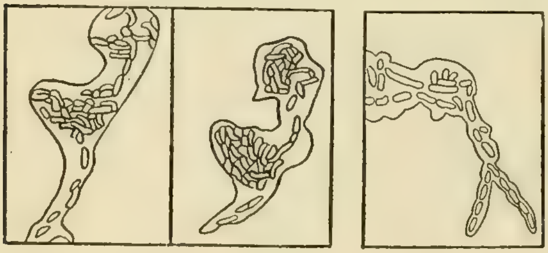
Fig. 36. Macrophage with hyaline protoplasmic process (*ps*) spreading over a *subtilis* chain (*s*). The latter becomes out of focus in the upper right hand corner of the picture.

Fig. 37. Two macrophages with *subtilis* chain (*s*) between them. Each cell has ingested one end of the bacterial chain and has extended a hyaline process (*ps*) on that portion which lies between the cells. The *subtilis* appears as a white chain and the two processes as delicate sheaths with dim outlines separated from the bacteria by dark spaces. The processes from the two cells met and remained approximated for some minutes; both were then withdrawn into their respective cells. One process was observed to extend again out over the *subtilis* chain before the field was lost to view.

In mixing the phagocytes, erythrocytes or bacteria and serum as described many collisions between phagocytes and test particles are brought about. Additional contacts between particle and phagocytes may later be made by the locomotion of the latter. In the absence of sensitizing serum the test particles typically neither stick to one another nor to the phagocytes (Figs. 1 and 2), and the particles are not ingested. In the presence of dilute sensitizing serum, agglutination of the particles and adhesion to the phagocytes may be much in evidence with little complete ingestion occurring (Figs. 5, 6 and 11-14). In the presence of more concentrated sensitizing serum, the test particles adhere to the phagocytes and are drawn into their cytoplasm in great numbers (Figs. 7-10, 29 and 30).

Text-Fig. 3

The test particles may be drawn into the phagocytes with comparatively little distortion of the latter (Figs. 29 and 30). Or a process composed of the hyaline superficial protoplasm may flow out over the surface of the particle undergoing phagocytosis (Figs. 3,4, 15-19, 28 and 34-37). A semidiagrammatic tracing of Figs. 15-19 is shown in Text-fig. 3. Or the spreading of the leucocytes over the sensitized particles may cause marked deformation of the leucocytes (Figs. 20-24 and 25-27). A semidiagrammatic tracing of Figs. 25-27 is shown in Text-fig. 4.



Text-Fig. 4

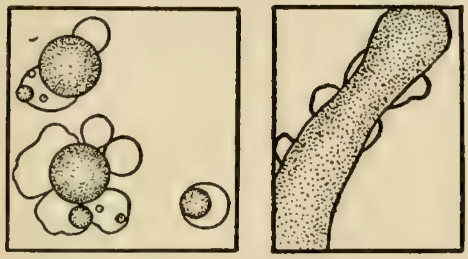
The types of ingestion described of course merge into one another. For instance four chains of sensitized *subtilis* bacilli were seen arranged in a diamond-shaped figure with a suspended spherical macrophage in their center. When first observed the chains were merely tangent and adherent to the macrophage surface. Gradually the areas of contact between *subtilis* chains and phagocyte surface increased, the adherent tangents becoming arcs of circles, which were slowly drawn into the macrophage protoplasm. The ends of the chains projected for a time beyond the macrophage surface, but these eventually were drawn in also; in several instances hyaline processes were observed to flow out over the projecting end of the *subtilis* chain as the last step in its ingestion.

For purposes of comparison between observation and deduction from theory the essential point is that in all instances observed the particles were not taken up in vacuoles of the suspending medium; on the contrary *the protoplasm of the phagocytes was in immediate contact with the surface undergoing phagocytosis.* Phagocytosis as observed, then, is primarily a phenomenon of surface spreading—the spreading of the phagocyte surface over the surface of the object undergoing ingestion. Prediction from theory and from experimental analysis is thus in agreement with observation on this second essential point.

Before leaving this point, however, two possible sources of confusion should be mentioned. Phagocytes of the large mononuclear type, already mentioned, are able to form delicate petal-like extensions of their peripheral hyaline protoplasm—the "sheet-like pseudopods" of Smith, Willis, and Lewis [43], the "undulating membranes" of Carrel and Ebeling [44], the "*pseudopodes pétaloides*" of Fauré-Fremiet [40]. Figs. 33 and 35). W. H. Lewis[45] has described under the term "pinocytosis," and shown in moving pictures, the engulfing of tiny vacuoles of the fluid medium by these processes. Should such a vacuole contain a minute particle it would of course be engulfed also. However, although we have seen the phagocytosis of a large number of bacteria and erythrocytes by direct extension of the phagocyte surface over the surface of the particle ingested, we have never observed ingestion in a vacuole. Phagocytosis and pinocytosis we believe to be quite different phenomena.

Another possible source of confusion is the fact that in stained films showing phagocytosis, bacteria can often be seen to lie in little vacuoles in the cytoplasm [43, 46]. These digestive vacuoles are seen especially about the bacteria which have been ingested for some minutes and have been moved in toward the center of the cell. These vacuoles are a phenomenon not of ingestion but of intracellular digestion.

Partial Ingestion. Fenn's formulation of surface forces in phagocytosis predicts that under certain conditions partial ingestion should occur; this is an important point of departure from the earlier formulations of Rhumbler[47] and Tait[48]. Fenn recognizes two conditions:



Text-Fig. 5. Tracing of Figs. 31 and 32. Macrophages white with black outlines; mineral oil stippled.

(a) The free surface energy is at a minimum when the particle is partially ingested; surface forces are therefore in equilibrium and satisfy the equation $S_1 = S_{12} + S_2 . \cos \theta$.

(b) Surface forces would tend to bring about complete ingestion, but this is prevented by the resistance to deformation of the phagocyte; surface forces are therefore not in equilibrium but are held in check by viscosity.

The second condition has certainly been realized in our experiments, and to the best of our belief also the first. Figs. 31 and 32 show fields in which macrophages were mixed with an emulsion of light California mineral oil. A tracing of Figs. 31 and 32 is shown in Text-fig. 5. Emulsion droplets of small size are readily and completely ingested by the macrophages. On the larger drops the macrophages spread (Figs. 31 and 32) to positions determined by the balance between surface forces and their own resistance to deformation.

Incidentally it may be mentioned that such small emulsion droplets are very readily ingested by macrophages, but ordinarily not by polymorphonuclear leucocytes. Such a difference cannot be explained by differences in resistance to deformation, since the polymorphonuclears are on the average more fluid cells than the macrophages. This is evidently an instance in which a difference in the surfaces of the two types of phagocyte is a critical factor in determining phagocytosis. Another such instance was found in the quantitative phagocytosis study;—collodion particles are readily ingested by macrophages but not by polymorphonuclear leucocytes [42].

Partial ingestion with surface forces in equilibrium is more difficult to demonstrate conclusively. When weakly sensitized erythrocytes are mixed with phagocytes partial ingestion often occurs (Figs. 5, 6 and 11-14). Such partially ingested cells are not completely ingested during the time they are kept under observation, even though this may be far longer than is required for complete ingestion of more strongly sensitized erythrocytes. It is difficult to believe that the partial ingestion by such a fluid cell as is shown in Figs. 11-14, could represent anything other than equilibrium under surface forces. Moreover in stained preparations [46] it has very frequently been observed that strongly sensitized bacteria were completely ingested, whereas weakly sensitized bacteria under otherwise similar conditions were merely adherent to the surfaces of the phagocytes. Although we realize that such observations fall somewhat short of rigorous proof that the surface forces are in equilibrium, we believe that this is by far the most probable interpretation. The third deduction from theory, namely the occurrence of partial ingestion, is thus likewise in agreement with observation.

Viscosity. L. Loeb[49] has related the amoeboid motion of the amoebocytes of *Limulus* to, "1) changes in consistency in the ectoplasmic layer as well as in the granuloplasm, 2) phenomena of contraction and 3) surface tension changes." Loeb in 1927 sought to carry over these conceptions to the explanation of phagocytosis by mammalian cells, assigning a primary importance to softening of certain parts of the surface layer of the cell in contact with a foreign body. Whether or not such local softening occurs on contact of phagocytes with foreign particles, it is evident that the quantitative correlation which has since been demonstrated between phagocytosis and the surface properties of the particles phagocytized is not explained by viscosity changes, and is explainable in terms of interfacial tension relations.

The resistance of the protoplasm to deformation is, on the other hand, a modifying factor in phagocytosis which, under certain conditions, may reach critical importance. Fenn[50], for instance, found very high temperature coefficients for phagocytosis below 30°C. as compared with those above 30°. He interpreted his data as indicating that below 30° the viscosity of the phagocytes was so high as to become the limiting factor for phagocytosis. Ponder[34] has also considered the effect of cytoplasmic viscosity as influencing the rate of ingestion. He has also dealt with the case in which movements in the surrounding fluid tend to dislodge particles which might otherwise be ingested, and has shown that either great cytoplasmic viscosity, or great turbulence of flow in the fluid, tends to prevent phagocytosis. This has since been verified experimentally [51].

An average difference in viscosity between phagocytes of the large mononuclear and the polymorphonuclear types has been observed by E. R. and E. L. Clark [52], by Goss [53], and by ourselves [54]. The macrophages offer, on the average, more resistance to deformation than the polymorphonuclears. This difference has been evidenced in our own study in two ways. In the first

place the act of ingestion is, on the average, more quickly accomplished by the polymorphonuclears, and in the second the polymorphonuclears are more readily distorted to all manner of bizarre shapes in spreading over the larger bodies phagocytized (Figs. 25 and 27).

Unformulated Factors. It seems clear then that surface forces are a principal factor in determining ingestion, and that viscosity is an important factor in controlling its rate. It is perhaps worth emphasizing, however, that a complete explanation of the behavior of phagocytes is not afforded by these factors alone. A particle phagocytized under the action of surface forces does not enter a homogeneous liquid, but a system possessing internal organization in high degree. The process which has spread over the particles undergoing phagocytosis is frequently retracted (Figs. 15-26). The protoplasm of the phagocytes possesses elastic properties (Figs. 11-14).* The ingested particles are commonly moved in toward the center of the cell. They frequently undergo rapid intracellular digestion. The formation and retraction of pseudopods appears to be the consequence of internal changes within the cell, as well as of the tendency of the cell surface to spread upon external surfaces. Reversible changes in viscosity, as evidenced by the appearance and disappearance of Brownian movement, may be seen to occur in local areas within the cell.

The phagocyte, then, is a complex system delicately responsive to internal and external influences. Interfacial tensions, and under certain conditions viscosity, are critical factors in determining the ingestion of particles with which the phagocyte has come into contact. Deductions from the formulation of these factors by Fenn and Ponder are in agreement with observation and with experimental analysis. However, other and still unformulated forces also enter into the behavior of these remarkable cells.

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Discussion

Dr. Abramson: In these observations of Fenn's does the specific effect of MnO_2 occur with each particle and every leucocyte, or does it happen once in a hundred? Is this a small percentage observation or is it a regular thing?

Dr. Mudd: I do not know the percentage of instances in which attraction of particle for leucocyte was demonstrable. But Fenn analysed his data carefully, and was convinced that in the case of manganese dioxide particles the contacts were more than could be accounted for by chance collisions.

Dr. Fricke: Were the suspending solutions in Fenn's experiments saturated with manganese dioxide?

Dr. Mudd: No.

Dr. Fricke: Would not saturation of the medium with manganese dioxide afford a method of testing the theory of chemical attraction?

Dr. Mudd: I should think it would.

Dr. Abramson: Did you say that serum albumin is not much adsorbed?

Dr. Mudd: If bacteria are suspended in the euglobulin, pseudoglobulin, or albumin fractions of unheated normal serum, phagocytosis is usually somewhat promoted in each case. But if the bacteria so treated are washed, the surface alterations, and phagocytosis-promoting effect, persist, at least partially, after the globulin treatment, but not after treatment with albumin. It would seem, then, that the albumin is less firmly adsorbed than the globulins.

Dr. Abramson: You mentioned that the leucocytes were able to phagocytize bacteria, even in the presence of such relatively high concentrations of aluminum ion as N/2000, and thorium, also, I believe. Do you think that the thorium was selectively adsorbed by the bacteria and did not poison the leucocytes?

Dr. Mudd: At least it did not poison the leucocytes sufficiently to prevent ingestion.

Dr. Abramson: I am thinking of it from this point of view: if you had the bacteria positively charged by thorium ions, and the leucocytes still remaining negative, you could account for that rather exceptional case of phagocytosis by the adhesion resulting from the difference in charge. Do you not think that is possible, even probable?

Dr. Mudd: Yes.

Dr. Abramson: In all your cases of phagocytosis, the bacteria and the leucocytes have the same charge.

Dr. Mudd: Yes, but wouldn't you, taking your case, expect the leucocytes to be reversed, too?

Dr. Abramson: Not necessarily. I would expect that if you have two types of surfaces, "a" and "b," there would be a difference in adsorption of polyvalent ions by "a" and "b." That might account, then, for the phagocytosis in the presence of the polyvalent cations.

Dr. Ponder: That would do it.

Dr. Abramson: It is a sort of cross-agglutination between the bacteria and the leucocytes. This case, of course, is analogous to the precipitation of one colloid by another, opposite in sign of charge.

Dr. Chambers: Your proposition presents the idea that protoplasm is of such a nature that when a particle on the outside is wetted by the protoplasmic surface, it will be drawn into the substance of the protoplasm. This may happen if the protoplasmic surface possesses a monomolecular oriented palisade structure of matter which is of the same nature as that of the interior, except for its oriented disposition.

However, there is another view, viz., that the protoplasmic body possesses a differentiated plasma membrane which is qualitatively different from the internal protoplasm. In other words, the plasma membrane is a layer of material with *two* interfaces, an external one, in contact with the aqueous environment, and an internal, in contact with the internal protoplasm. Evidence for this is suggested by (1) the high electrical resistance of cells, and (2) the impermeability of the surface, both from without and from within, to non-penetrating dye solutions, e.g., phenol red.

I would postulate that surface tension forces, existing at the interfaces of the plasma membrane, are of different orders, the interface in contact with the aqueous environment possessing a higher tension than the interface in contact with the heterogeneous, but freely water-miscible, internal protoplasm.

The particle being ingested might then be wetted by the plasma membrane at its external interface and be pulled in, in the manner you suggest. Owing to the relatively high tension of the external interface, this interface would tend to develop an even contour, with the consequence that the particle would form a protrusion of the plasma membrane along its inner interface. The very low forces at play along this inner interface may result in the continued formation of myelin-like protrusions, into the internal protoplasm, which

pinch off periodically, one of them carrying along the particle with it.

This is not a far cry from the formation of food vacuoles by certain protozoa. In short, the object being ingested is wrapped or infolded within plasma membrane material, and in this condition is pinched off into the interior of the cell.

Dr. Mudd: In the formulation of surface forces in phagocytosis no assumption is made as to whether the cell surface is a monomolecular film or a thicker layer, except that it is implicitly assumed that the cell surface is not significantly altered in spreading over the particle phagocytized.

Kite described the mammalian leucocyte as naked protoplasm. Our observations agree with this, in that no microscopically visible membrane, such as can be seen on the erythrocyte, can be made out even with cardioid condenser and oil immersion objective. Of course, however, I believe that we must postulate a differentiated surface film to account for impermeability and other surface effects.

We have never seen with dark or bright field illumination, nor received an account of such myelin-like protrusions into the interior of the phagocyte, although they may often be seen on the external surface. Moreover, the pinching off of protrusions carrying particles with them suggests, to me, particles small in relation to the dimensions of the phagocyte. We have often watched leucocytes spreading over and phagocytizing chains of bacteria considerably longer than the diameter of the resting leucocyte.

* * * * *

The idea of negative surface tension has recently appeared in the literature on amoeboid cells and phagocytosis; discussion of this idea by this group would be most desirable. The idea was introduced by Leathes in treating the myelin forms of lecithine in the following passage:

"Lecithine is a fat containing two fatty acid radicals instead of three, one of them certainly unsaturated, the other probably saturated; in place of the third there is phosphoric acid condensed by one of its hydroxyl groups with the glycerol and by a second with a basic alcohol, the third hydroxyl group being free . . . .

"But though lecithine is a fat and in a film behaves as one, lecithine in bulk behaves very differently from a fat or oil in contact with water. The 'surface tension" of oil against water is such that the number of molecules of either which are allowed by the attractions of other molecules in their neighborhood to remain in the surface is kept as small as possible, that is to say, the surface area is kept as small as possible. A drop of oil suspended in water remains spherical. With lecithine it is just the other way. The number of molecules of lecithine in contact with water tends to increase and to become as large as possible; that is why the surface is protruded into the water and the myelin forms appear. The attraction exerted by water on that part of the molecule of lecithine by which it differs from a simple fat is so great that the molecule is drawn to and held by the water more strongly than it is by the other lecithine molecules in its neighborhood. This attraction prevails over the attractive forces between the paraffin chains, partly because these can not be packed so close as in a triglyceride. The cohesion between the lecithine molecules is consequently weakened at a water surface, and the surface tends to grow and not to be kept small; if the term surface tension must be used, the surface tension is negative." (The Lancet, 1925, **208**, 960).

Fauré-Fremiet, in his beautiful studies of the surface hyaloplasmic processes of invertebrate amoebocytes, brought out many points of resemblance of these processes to the myelin forms of lecithine, and adduced evidence to indicate that the formation of these processes was probably dependent on the presence of lecithine in the surface protoplasm. He adopted Leathes' conception of negative surface tension.

"Concerning the hyaloplasmic processes (petaloid pseudopods) we can now imagine that following the schema suggested above, their formation results from a local swelling, by imbibition, of the hyaloplasmic zone; we shall make no hypothesis as to the cause of such localized variations, but we shall recall that our measurements of refractive index and volumetric calculations are favorable to this idea. In this case all the properties of the hyaloplasmic processes, their negative surface tension, their lamellar expansion, indicate an anisotropy in the distribution of capillary tension, make us think of a swelling of lecithine in the presence of aqueous solutions and of the formation of myelin figures of a special type." (Protoplasma, 1929, **6**, 603).

Most recently Volkonsky has taken up the idea of negative surface tension, and has tried to rewrite Dr. Ponder's equations for the conditions of phagocytosis, giving the cell-plasma interfacial tension a negative value. The predictions drawn from Volkonsky's treatment are the opposites of the correct ones. He then justifies his predictions by making interpretations of the experiments of Mrs. Mudd and myself which are quite unacceptable to us. (Bulletin Biologique de la France et de la Belgique, 1933, **67**, 168).

Dr. Ponder: One of the most interesting points for discussion concerns Volkonsky's idea that the cell-plasma interface may have a negative surface tension. The conception of negative

surface tension is becoming dangerously prevalent among biologists, and so I want to make four points about it, and then leave the matter to discussion.

The first point is that the lowness of the cell-plasma interfacial tension, even if we admit that it is very low, is not an essential point, for the equations which deal with the conditions for ingestion are not concerned with the cell-plasma interfacial tension in absolute units, but with the relative values of three interfacial tensions. If one allows that the cell-plasma tension is low in absolute units, I do not see why one should not imagine the particle-plasma and cell-particle tensions to be of an equal order of lowness. It is a logical fallacy to think of a tension of 0.5 dynes/cm. as "virtually zero"; compared with the other interfacial tensions existing in the system, 0.5 dynes/cm. may be a very considerable value.

In the second place, Volkonsky is not content with a small tension at the cell-plasma interface, but makes the tension negative. This, of course, turns everything upside down, and the condition for ingestion becomes the condition for no ingestion, or *vice versa,* for he changes the sign of S_2 from positive to negative. His idea seems to be that one can properly make a very small value equal to zero, and then make zero equal to a negative quantity.

Thirdly, even if the cell-plasma tension were negative, I am sure that the equilibrium condition would not be given by $\cos\theta = (S_1 - S_{12})/-S_2$, as Volkonsky supposes. The equilibrium condition, if any, would have to be worked out from first principles, and not merely by changing a sign.

Lastly, I simply do not know what a "negative surface tension" is. In Leathes' idea of negative surface tension it is not contended, I suppose, that the volume of the myelin forms remains constant as their surface expands, and I know of no evidence against the expansion of surface being really due to an imbibition of water by the lecithine. It seems to me that if one has a surface which is going to expand continually because of a "surface tension," one will have a surface which will disintegrate and undergo spontaneous emulsification.

Dr. Abramson: I can conceive of a negative surface tension exerted under these conditions. Take a stable particle in a liquid and let the interfacial tension be very small. Charge the surface of the particle so that the opposing force exerted by the charge will be greater than the force of surface tension.

Dr. Ponder: That is essentially Ostwald's old model of a charged and liquid surface which would break into a fountain.

Dr. Cole: It may be of interest to point out that under certain conditions, instability occurs before a net or effective surface tension is reduced to zero. When a spherical droplet is electrically charged, the mutual repulsions of the charges exert a force which acts against the component of surface tension directed inward. The electric potential necessary to reduce the force normal to the surface to zero may be computed easily. A complete analysis shows, however, that the droplet will become unstable and separate into two at one-half the value of potential necessary to "reduce the surface tension" to zero.

Dr. Ponder: I have been interested to see the pictures of the rod-shaped bacteria being ingested in an end-on position. You will notice that this is the condition for θ being single-valued, and it seems to be the position in which the bacillus ought to go in. Does a rod-shaped particle always go in end-on?

Mrs. Mudd: Bacterial rods after contact with the leucocyte are often seen to be oriented so as to be ingested end-on. This has been the more usual method of ingestion in our observations, although occasionally the bacterium may be taken into the leucocyte with its long axis tangent to the leucocyte surface.

Dr. Cole: If it has not already been tried, it should be interesting to investigate the phagocytosis of air bubbles where the surface tension is high.

OSMOTIC BEHAVIOR OF RED CELLS. II.

Eric Ponder

In my last lecture I dealt with the applicability of the Boyle-Marriotte law to the volume changes observed when red cells, and probably most other types of vertebrate cell, are immersed in hypertonic or hypotonic solutions, and concluded that the law does not apply in its simplest form, but that the cells shrink in hypertonic solutions, and swell in hypotonic solutions, as if part of their contained water were "bound," or, alternatively, as if osmotically active substances were exchanged between cell and environment. Now if the medium is sufficiently hypotonic, the swelling of the red cell is so great that it bursts and liberates its haemoglobin, and so some light can be thrown on the foregoing conclusion by considering the relation between the volume of the cell and that extreme hypotonicity of the medium in which haemolysis occurs. The matter can best be approached by seeking the solution to two classical problems:

1. Why do the erythrocytes of the same animal (e. g., the rabbit) haemolyse in quite different equivalent concentrations of different substances, e. g. in 0.52 p.c. NaCl, but in a glucose solution whose osmotic pressure is equivalent, not to a 0.52 p.c. NaCl, but to a 0.38 p.c. NaCl?

2. Why do the red cells of one animal (e.g., man) haemolyse in a 0.40 p.c. NaCl, while those of another animal (e. g., the sheep) haemolyse in a solution of a different concentration, e. g., 0.56 p.c. NaCl?

I may remark at the outset that there is no doubt about the experimental facts, and that both problems have well known practical applications associated with them. The comparison of the concentrations of different solutions in which erythrocytes just begin to haemolyse is one method of finding the so-called "isotonic coefficients," and the observation of the concentration of the solution in which different types of cell, or cells from the same kind of animal under different conditions, just begin to haemolyse is the method used for determining what is commonly known as "red cell fragility," a property of some clinical and general biological interest.

The generally accepted explanation for hypotonic haemolysis, introduced by Hamburger and supported by the investigations of Koeppe, Ege, Warburg and Winge, Jacobs, and many others, is that the cells take in water, swell, and finally burst or leak pigment because their membranes become so stretched. Some observers have postulated the existence of "bound water," while others have treated all the water as "free"; such postulates influence the computation, but the general idea is the same for all. It is always assumed, however, except by those few observers who look upon the lysis as a result of imbibition, etc., that the volume of the cell is calculable from simple osmotic laws, "bound water," perhaps, having to be taken into account.

Now if this is so, it is very difficult to account for the fact that the red cells of a given animal undergo lysis in such concentrations of different substances as are quite different from an osmotic standpoint. If simple osmotic laws are sufficient, even if we allow a constant quantity of "bound water" to be present, it is hard to see why rabbit red cells, for instance, should haemolyse in 0.52 p.c. NaCl but in glucose equivalent to 0.38 p.c. NaCl, for the two concentrations should be the same and not different. Moore and Roaf have rejected the entire osmotic theory because of this difficulty, and have fallen back on a rather indefinite hypothesis which accounts for haemolysis as the result of the imbibition of water by a dense intracellular stroma. The difficulty is usually explained, however, by introducing subsidiary assumptions, some of which are the following.

1. Ege has considered the possibility of the dissociation of osmotically active substances within the cell changing when the erythrocyte is immersed in solutions of different substances, but he dismisses the explanation on the ground that the possible changes are too small to account for the observed phenomena. I have no doubt that we could account for small differences in this way, and also by taking account of possible pH changes, but the observed differences are far too large.

2. One might suppose that the immersion of the cells in solutions of different substances, e.g., NaCl, glucose, or sucrose, brings about an alteration in the quantity of "free water." The amount of this substance would have to be different for almost every different suspension medium, so the explanation would not be a very good one even if "bound water" existed in appreciable quantities; as it does not, we need not consider this explanation further.

3. The next explanation is that the erythrocyte has a membrane which is more resistant to stretching when in glucose (say) than when in NaCl, and not unlike this hypothesis is one which depends on Brinkman's observations [1] [2], (now disproved[3] that the fragility of the cell depends on the lecithin/cholesterol ratio in the membrane; different substances, affecting the ratio in different ways, might conceivably cause such differences in the membrane as would allow it to stand

more stretching in a solution of one substance than in a solution of another.

Now this idea is one which can be tested experimently, for we can measure the volume at which the erythrocyte begins to haemolyse in solutions of different substances, and we can then tell whether the surface is really more stretched in a solution of a substance such as glucose than it is in one of NaCl. The cells are placed in solutions of NaCl of descending degrees of tonicity, and similarly in a series of solutions of glucose. In a certain concentration of NaCl, and in a certain solution of glucose, commencing lysis is observed, and the volume of the cells in these two solutions is then determined diffractometrically. The experiment is easy to do, and the result is always the same, allowing, of course, for the small errors inherent in the method: the cells begin to haemolyse at the same "critical volume," irrespective of the nature of the substance in the surrounding medium. There is, of course, a little variation from animal to animal, and more in some animals than in others, but, as an instance, the rabbit erythrocyte, the normal volume of which is about $58\mu^3$, shows commencing lysis when its volume has increased to about $78\mu^3$, irrespective of whether the surrounding medium is hypotonic NaCl, KCl, glucose, sucrose, or any one of the monovalent chlorides. The existence of this "critical volume" was first recognized by Jacobs[4], and is a very important point.

4. This being so, we can proceed in the following way. Let us regard the cell as an imperfect, instead of as a perfect, osmometer, and let us again measure the degree of imperfection, or the amount of leakage of osmotically active substances, in terms of the fiction of "bound water." Then we can write as a general equilibrium condition

$$\frac{11.0\ (Q_1)\,.\,(R/100)}{(Q_1)\,.\,(R/100) + v} = \frac{T}{100 - v} \qquad (1)$$

in which all the symbols have the same meaning defined in the last lecture, i. e., T the tonicity in which a percentage increase in volume v occurs, Q_1 the total water in the cell, and R the ratio Q_2/Q_1, where Q_2 is the quantity of water which would have to be assumed to be contained in the cell if it could be treated as a perfect osmometer. Now this expression is true for all sorts of values of T and v, and so we can immerse the cells in solutions of different tonicities and measure v for each; then remembering that Q_1 is constant, we can use the results to give a series of equations from which we can get a "best value" for R. Having obtained this for a series of solutions of NaCl, (say), we can insert in the expression the proper value of R and of Q_1; we then have a numerically soluble equation in T and v, applicable to systems containing red cells suspended in NaCl of various degrees of tonicity.

Next we can do the same thing for the cells suspended in a series of hypotonic solutions of another substance, say glucose, and obtain in a similar way a numerically soluble equation in T and v for this system. If this is done, the first thing which strikes one is that the "best values" for R for the system of cells in hypotonic NaCl is much higher than that for the system of red cells in glucose, the former being usually about 0.5, and the latter being more nearly 0.25. This, of course, is not surprising, for all that it means is that the leakage of osmotically active substances is greater in NaCl than in glucose, which is generally admitted. But we can go a step further, and use the equation in the following way.

Let us suppose that we find by experiment that in a solution of NaCl of tonicity 0.52 we get commencing lysis, and that in such a solution the volume of the cells is 135 p.c. of their normal volume. Then remembering that this is the "critical volume," and that it is the same when the cells are in glucose as when they are in NaCl, let us insert the proper value for Q_1, the value which we have just found for R for the systems of cells in glucose, and the same value of v as found for the NaCl systems, in expression (1), and let us solve for T, i. e., from the value of R for the glucose systems and from the value for the "critical volume" found for the NaCl systems, let us predict the tonicity of glucose in which the cells will just undergo lysis. The result will only be correct if the assumptions and measurements are correct, and it is surprising how closely the prediction can be made, not only for systems of cells in glucose, but for systems of cells in quite a variety of hypotonic solutions. The following table shows one series of results: [5]

Substance	Tonicities used for finding R.	Predicted tonicity for lysis	Observed tonicity for lysis
NaCl	0.7, 0.6, 0.54	0.52	0.52
KCl	0.78, 0.76, 0.7	0.62	0.66
LiCl	0.9, 0.7	0.67	0.63
Glucose	0.66, 0.5, 0.46	0.38	0.36
Sucrose	0.08, 0.06	0.52	0.50
KNO_3	0.8, 0.7, 0.6	0.59	0.59

Considering the errors inherent in the methods, these results are quite striking, and the puzzling fact that red cells haemolyse in solutions which are far from osmotically equivalent can be quite well explained by assuming that the cells undergo lysis when they reach a certain critical volume, constant for all these substances, but that the leakage of osmotically active substances, as shown by the value of R, is different

in the case of each. That this hypothesis is adequate is shown not only by the fact that direct experiment reveals the same critical volume and the different values of R, but by the fact that by taking account of these two factors alone we can predict with considerable accuracy the tonicity of each substance in which lysis will begin to occur, even although there are wide differences in the concentrations which bring about haemolysis.

Before passing to the next problem, I may remark upon the rather curious fact that the cell is "most fragile" or "least resistant" in hypotonic solutions of the very substances in which it loses osmotically active substances least, and "most resistant" in solutions of substances in which the leakage is great. The most resistant cells are thus the least "perfect" from the osmotic standpoint, and, far from their membranes being peculiarly "strong" or "extensible," as seems to be the general idea among clinicians and biologists, they are peculiarly liable to leak and to lose cations into the surrounding fluid.

We have now to consider the second classical difficulty, that of explaining why the cells of one animal are "fragile" and easily haemolysed by hypotonic solutions of a particular substance, e.g., NaCl, whereas those of other animals are "resistant" and can be haemolysed only by much more hypotonic solutions. There are considerable differences among the cells of various animals in this respect; those of the sheep and ox, for instance, haemolyse in NaCl solutions of about 0.72 to 0.66 p.c., whereas those of the rabbit and man are much more resistant and haemolyse only when the tonicity of the medium falls to about 0.40 p.c. There are, of course, considerable differences also between the cells from different animals of the same species, but, taking average values, the types of cell can be arranged in a series known as the Ryvosch Series[6], which runs: man, guinea-pig, rat, rabbit, dog, pig, cat, ox, goat, sheep,—the most resistant kind of cell occurring first. The subject of this different fragility has been widely studied, but the only two suggestions of any consequence which have been advanced are that of Brinkman and van Dam, who regard the fragility as dependent on the lecithin/cholesterol ratio in the membranes of the different types of cell, and a suggestion that the fragility is a function of cell diameter, the larger cells being stated to be, in general, the more resistant. Brinkman's hypothesis has been contradicted, and the theory that the fragility is determined by diameter will not stand very close examination, although, as we shall see, it has an element of truth in it.

The problem of accounting for the differences in fragility is a very complicated one, for there are at least four factors upon which the fragility must necessarily depend. (1) Even if the red cell were a perfect osmometer, the concentration of NaCl in which it would haemolyse would depend on the critical volume which it could attain, for the greater this volume, the less would be the concentration of NaCl which would bring about lysis, other things being equal. The critical volume, of course, might vary for the cells of different animals. (2) Even if the cell were a perfect osmometer, the swelling in any hypotonic solution of NaCl would depend on the quantity of water which the cell contained, and the smaller the amount of water the greater would have to be the degree of hypotonicity of NaCl which would bring about swelling to the same critical volume. (3) Still regarding the cell as a perfect osmometer, the swelling which it would undergo in any given tonicity of NaCl would depend on the osmotic pressure of the cell interior, and this is not the same for the cells of all mammals. (4) And lastly, if the cell is an imperfect instead of a perfect osmometer, the extent to which it would swell, and therefore the tonicity of hypotonic NaCl in which it would reach its critical volume, would depend on the extent to which it could lose osmotically active substances, i.e., on the value of R.

Our problem is to decide which one of these factors is responsible for the different fragilities of the cells of different mammals, and a very difficult problem it is, particularly when one considers that not one factor alone, but several acting in conjunction, might be responsible for the result. But even a roughly quantitative explanation is better than none, and so we shall see how far our experimental methods will take us.

The quantity of water present in the cell is easily determined, so factor (2) offers no difficulty, but the osmotic pressure of the interior is much more difficult to find. Assuming that it is the same as that of the surrounding plasma, we might determine it by finding the depression of freezing point of the latter, but a far more convenient way is to call the tonicity of the plasma unity, and that of the cell interior unity too. This gets rid of factor (3) immediately, but determines that all the experiments are to be carried out in the hypotonic plasma of the animal whose cells are concerned. This, perhaps, is more advantageous than otherwise, for pH changes are much less liable to affect the results when the cells are suspended in plasma than when they are suspended in saline.

As soon as we decide to use hypotonic plasma as the suspension medium our choice of methods for measuring red cell volume becomes severely limited. The colorimetric method is useless for determination of cell volume in solutions in which lysis is likely to occur, the haematocrite

method is out of the question, and diffractometric measurements cannot be made, for the cells are discoidal. We are therefore left with the methods which determine percentage increases in cell volume from changes in cell density, changes in haemoglobin content, or changes in the water content of the cells after swelling[7], and, as the last mentioned is the simplest, we can select it for the determination of the relative increases in volume which the cell shows when immersed in hypotonic plasma of various degrees of tonicity, and for the evaluation of R. This disposes of factor (4).

Lastly, we have to find the critical volume at which the cells haemolyse (factor 1), and here we encounter a difficulty which can only be overcome by the use of a special method. The methods which measure swelling from changes in water content, density, and haemoglobin concentration are useless, for they do not measure volume in absolute units, but only the swelling which would occur if the cell were a perfect osmometer (which it is not) containing a quantity of "free water," $Q_2/Q_1 = R$, (which it does not). The fiction of "bound water" is thus used as a measure of the fictitiousness of the idea that the cell is impermeable to cations, and volume is not measured directly at all; consequently these methods are useless for the determination of the critical volume, which must be found in absolute units. The colorimetric method is excluded, for the same reason as mentioned above, and haematocrite methods are too unreliable; we have therefore to determine the critical volume in hypotonic NaCl solutions diffractometrically, and to be content with the result, although the effect of factor (4) is measured in hypotonic plasma.

But this does not end the difficulty, for what we are interested in is not the critical volume *per se*, but the percentage of the normal volume to which the cell will swell, attaining its critical volume, before it begins to lose pigment. We have therefore to measure the normal volume in addition. Now we might do this colorimetrically, but the technical difficulties of making colorimetric determinations of volume side by side with all the other determinations is too formidable a task to be attempted. We can, however, avail ourselves of a very neat method of measuring cell volume in undiluted plasma. If a small amount of lecithin is added to the plasma, and cells added thereafter, the cells become perfectly spherical without change in volume, and diffractometric measurements of volume can be made in a few minutes. To find the percentage of the normal volume to which the cell will swell without haemolysing, we accordingly find the normal volume diffractometrically in lecithin-treated plasma, and the critical volume in a hypotonic saline: divison of the latter figure by the former then gives us what we require, the maximum increase in volume which the cell can undergo while still remaining intact.

I can pass over the details of the methods employed, and go at once to the results. There is little point in making determinations of the effects of all the factors for all the types of cell in the Ryvosch Series, for the differences of resistance between some of them is very small; I shall therefore refer to the results for the cells of man, the rabbit, the sheep, and the ox, ignoring the remainder in the meantime.

The determinations of the value of R for these various types of cell, each immersed in the hypotonic plasma of the same animal from which it was obtained, leave little doubt that R is essentially the same for all. It varies between 0.5 and 0.7, but the variation between the values for the cells of individual sheep, for example, is as great as is the variation between the values for sheep and rabbit cells. If a very large number of experiments were carried out, some differences, in a statistical sense, might emerge; after carrying out some twenty experiments or so, I am left, however, with the very strong impression that whatever factor may account for the different fragilities, it is not differences in the extent to which the different types of cell lose osmotically active substances. The cell of one type of animal seems to be just about as imperfect an osmometer as is the cell of another.

Similarly there is nothing striking about the quantity of water present in the cells; there are differences, but they are as great between cells of individuals of the same species as between cells of different types. Nor does the figure obtained for the total amount of water contained in the cell seem to have much to do with the figure obtained for the tonicity of plasma in which the cells haemolyse; again, large numbers of experiments might indicate some effect, but the effect is certainly not at all prominent.

It is a very different matter when we come to consider the critical volume at which the cells of the different types undergo haemolysis, for one immediately observes that the more resistant types of cell, e.g., those of man and the rabbit, assume far greater critical volumes than do the cells of the ox and the sheep, which are much more fragile. Calling the normal volume in plasma 100, and taking average figures, the cells of the sheep haemolyse at the critical volume of 126, those of the ox at 130, those of the rabbit at 137, and those of man at 146. There are, of course, individual variations, for the critical volume for rabbit cells may vary between 134 and 138, and that for sheep cells between 124 and 128; the differences, nevertheless, are unmistakable.

We therefore arrive at the conclusion that the factor mainly responsible for the different fragil-

ities of the cells of different animals is that different kinds of red cell are able to assume different critical volumes, the more resistant cells, such as those of man and the rabbit, being able to withstand much more distention and stretching of their membranes than are the less resistant cells of the ox or sheep. Other factors, such as the water content, the initial osmotic pressure of the cell interior, or the extent to which the cells can lose osmotically active substances, may contribute to the final result, but their effect is of secondary importance. The fragility of the cells of the various kinds of mammals is thus determined by a factor quite different from that which determines the resistance of any one kind of cell to hypotonic solutions of various substances; in the first case the most resistant cells are actually those which can withstand stretching of their membranes most, while in the second case it is the extent to which the cell can adapt itself to its environment by losing osmotically active substances, and not the extent to which the membrane can be stretched, which determines the result.

In conclusion, it is interesting to work out the extent to which the membranes of the different kinds of cell can be stretched before they either rupture or become permeable to haemoglobin. Taking average figures, we have:

Animal	Normal area	Stretched area	Extension ratio
Sheep	$46\mu^2$	$54\mu^2$	0.18
Ox	$60\mu^2$	$72\mu^2$	0.19
Rabbit	$72\mu^2$	$89\mu^2$	0.24
Man	$94\mu^2$	$121\mu^2$	0.28

All these figures for area, of course, refer to the cells in their spherical form. The table shows that the membranes of the cells which have the greatest resistance can be extended to a greater extent without rupturing than can those of the less resistant cells, for the extention ratio rises from 0.18 to 0.28, a very considerable increase.

At first sight it is difficult to find an explanation for this fact, unless we fall back on the very unsatisfactory hypothesis that the structure of the membrane of the human cell, for instance, is different from that of the membrane of the sheep cell, and that this difference, whatever it may be, results in the greater extensibility of the former. The whole matter becomes more comprehensible, however, if we put down, side by side, the figures for the volumes of the cell and for the absolute increase in area which can occur before rupture:

Animal	Volume	Increase in area	dA/V
Sheep	$30\mu^3$	$8.4\mu^2$	0.28
Ox	$44\mu^3$	$11.4\mu^2$	0.26
Rabbit	$58\mu^3$	$17.3\mu^2$	0.29
Man	$86\mu^3$	$26.7\mu^2$	0.31

We thus have the relation

$$dA/V \quad \text{or} \quad dR/R^2 = \text{const.}$$

to within a very small degree of error.

It is interesting to speculate on the meaning of this relation, and to do so we shall think of the cell membrane as a fluid or semi-fluid film, the permeability of which is governed by the properties of a few layers of molecules, the thickness of this layer being of the order of 0.01μ, and much thinner than the "morphological membrane" as a whole. This conception is the one which we have to arrive at if we consider Fricke's results for the capacity of the layer which prevents the migration of ions, and is quite in keeping with all that is known about the membrane. Now let us stretch the membrane as a whole, and with it the thin layer just referred to, and let us take account of the hypothesis advanced by Osterhout, that substances can be drawn from the interior of the cell (or from other parts of the membrane, whose volume is probably proportional to the cell volume) in order to augment, or even repair, the cell surface. Then we shall have what we may call a "reserve" of material available to be drawn into the thin layer when the latter is stretched, and the quantity of this will be proportional to the cell volume, or, more strictly, to the volume of the membrane as a whole. As the thin layer is stretched, it will be thinned even further, but the "reserves" will be able to make good the deficiency until they are exhausted. The greater cell volume, and the greater the quantity of these reserves, the more will we be able to stretch the thin layer without rupturing it, and this will give us the relation between increase in area and cell volume just referred to. Further, during the stretching of the thin layer and its continuous repair by a redistribution of the "reserves," it is not unlikely that the layer should become partially permeable to small ions, and this would explain the continuous loss of cations which has been the subject of these lectures.

This theory, of course, is highly speculative, but it at least explains the observed facts in a way consistent with what we know about the properties of the membrane and the possibilities of its repair.

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Discussion

Dr. Cohen: If cations are lost from the cells, would they not be lost in sufficient quantity to enable them to be determined quantitatively in the external medium?

Dr. Ponder: That has been done for muscle, and an excellent correspondence found. In the case of the red cell, it is not quite so easy to do. But it is easy to show that cations are lost into such a medium as hypotonic glucose, either by analysis, or by conductivity measurements.

Dr. Blinks: Have you, or Dr. Fricke, measured the capacity of cells which are swollen?

Dr. Ponder: We have done so, and it is the same as in the normal cell, per unit area.

Dr. Blinks: That would show that the membrane does not undergo thinning.

Dr. Ponder: Exactly. When its area is increased it is not increased by thinning, but by addition of the substances which I call "reserves".

Dr. Blinks: How long may a definite amount of swelling be maintained?

Dr. Ponder: Once a particular volume is attained it is maintained for hours.

Dr. Harris: There being differences in the haemolysis curves for different animals, data in terms of the volumes attained at 50 p.c. haemolysis, instead of at the beginning of lysis, might give different results?

Dr. Ponder: Yes, but not substantially different, for, although the percentage haemolysis curves, for the different types of cell, show slightly different scatter, the differences in scatter are not very great. Indeed, there are as great differences between ox and ox, say, as there are between ox and rabbbit.

END OF COLD SPRING HARBOR SECTION

HETEROCHROMATIC RADIATIONS AND EARLY AMPHIBIAN DEVELOPMENT

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In dealing with radiations of the Amphibian egg within the limits of the solar spectrum we are immediately concerned with the three principle light factors, namely: luminous intensity, quality, and radiant energy. This is particularly true of the Frog's egg which has an abundance of black pigment and therefore represents the theoretically perfect, non-selective absorption medium.

The first factor, luminous intensity, refers only to light of the visible spectrum and is the density of luminous flux per unit area known as the International Candle. Foot-candles by definition refer to composite candle-light, never monochromatic light, and may be measured by photronic photoelectric cells which are sensitive to total luminous intensity of a specific light source. According to Cady: "Luminous flux is the rate of flow of radiant energy evaluated with reference to visual sensation" and according to Trotter: "Photometry" (which is the method of measuring illumination) "is not measurement of an external or objective dimension or force but of a sensation." Here, then, we are dealing with a subjective factor which has interest from the standpoint of special sense organ physiology. The illumination factor need not be considered apart from the sense organs adapted to respond to luminous intensities. Experimental evidence for this is here presented.

The second factor, quality or wave-length, is measured by the spectrophotometer. Colored organisms cannot absorb light of the same color and yet the literature is abundant with references to the specifically lethal effect of monochromatic green on green plants, and the beneficial effect of violet, blue, and red on the same plants. Yung has reported similar results with the Frog tadpoles which evidence we are to examine here.

The third factor, radiant energy, is measured either with a thermopile or by the paired thermometer method. In either case the blackened radiometer is non-selective, being sensitive to all wave lengths of the radiant energy spectrum. In this work such relatively great amounts of energy were involved that the paired thermometer method was used. By this method the direct sunlight at zenith in April (the normal breeding season of *Rana pipiens*) registers about 25° C. In absolute units 1°C. by this method represents approximately 0.2 gm. cal. per sq. cm. per minute.

As a specific example of the distinction between various light factors, we can by direct experiment show that 3 cm. of distilled water will not reduce the luminous transmission from a G. E. –CX lamp in the slightest but will reduce the transmission of radiant energy by 48%. Radiant energy, then, is a physical factor characteristic of

CORNING GLASS FILTERS

Number	Spectral Range	Color	Rel. Luminous Trans.	Rel. Energy Trans.
#241	6560-7060	H. R. Pyr. red	14% ft. candles	62.10% C°.
#243	6200-7000	Red	25% "	66.94% C°.
#254	1u-3u	Infra red	00% "	59.19% C°.
#396	4000-6600,2.5u-	Light Alko	38% "	9.86% C°.
#586	3300-3900	Violet Ultra	00% "	9.80% C°.
#774	3650-3u	Pyrex	95% "	91.65% C°.

(Data on other Corning Filters may be secured through the author).

E. YUNG'S LIQUID FILTERS

	Spectral Range	Color	Rel. Luminous Trans.	Rel. Energy Trans.
Aq. gent. viol.	4200-5000 6600 plus	Violet	31% ft. candles	45.5% C°.
Alc. lyons bl.	4200-5800, 7000 plus	Blue	14% "	43.0% C°.
Aq. Nic. Nitr.	5000-6000	Green	10% "	4.4% C°.
Aq. K-chrom.	5200-7600 plus	Yellow	78% "	39.4% C°.
Alc. fuchsine (basic)	6400-7600 plus	Red	30% "	41.0% C°.

(In Yung's experiments the filters included two thicknesses of glass which reduced energy transmission but not luminosity. The absorption of 8 mm. of clear glass was 14% of the radiant energy).

radiations from the shortest gamma rays to the longest Hertzian waves while luminous intensity refers only to the narrow limits of human visibility.

The following table gives the transmissions of some of the filters used, distinguishing between luminous intensity, quality, and radiant energy. It will readily be seen that there is no correlation whatever.

Using the Bunson-Roscoe Law where K = IT and referring to I as intensity in terms of centigrade degrees on the thermometer and T as time in minutes, the value for K was kept uniform except where otherwise stated. This meant, for instance, that radiations under monochromatic green were twice as long as exposure under the red because of the particular energy transmissions of these two filters.

From the standpoint of the egg or tadpole there are other factors to be considered. Water alone absorbs radiant energy and because of its specific absorption tendency toward the red and heat rays, radiation of the Frog's egg must be without intervening water. Further, since Frog jelly is 78% water, we would expect that the jelly would be a protective layer against the heat rays. By direct experiment this proves to be the case. Also, the efficiency of the black pigment toward the various light factors should be considered.

The following data indicates that as long as K (radiant energy x time) is constant the growth rate of tadpoles will be the same regardless of the wave lengths or luminous intensities used. The eggs and tadpoles were placed in blackened finger bowls immersed in running constant temperature bath and water removed until the upper surfaces of eggs or tadpoles were exposed during radiations. Between radiations, eggs or tadpoles were placed in large containers with compartments separated by wire, so that there was diffusion continuity to equalize any food or metabolic conditions.

RANA PIPIENS

Filter	Spectral Trans.	Lum. Tr.	Energy Tr.	Exp. Min.	K	15 days Length (mm)		30 days Length (mm)	
Dark	00.00	00.00	00.00	00.00	00.00	15.50	0.00	22.00	0.00
#774	3650-3u	95.0%	91.65%	9.74	125.0	15.58	+0.08	22.10	+0.10
#554 bl.	3900-4900	4.0%	24.05%	24.75	125.0	15.52	+0.02	22.00	0.00
#401 gr.	4800-5800	3.0%	20.04%	29.69	125.0	15.50	0.00	22.14	+0.14
#349 y-r	5500-7000	52.0%	61.92%	14.41	125.0	15.52	+0.02	22.10	+0.10
#243 red	6200-7000	25.0%	66.94%	13.34	125.0	15.54	+0.04	22.00	0.00
#254 ir.	1u-3u	00.0%	59.19%	15.08	125.0	15.60	+0.10	22.12	+0.12

K = IT where I is intensity in terms of centigrade degrees and T is time in minutes. (Bunson-Roscoe Law).

K = centigrade minutes as measured by the paired thermometer method. Direct zenith midsummer sun registers about 32°C. and corresponding midwinter sun about 19°C.

Experimental data presented led to the following conclusions:

1. Frog's eggs, sperm, and zygotes without jelly show essentially the same limits of tolerance of total direct sunlight.

2. While the Frog jelly is permeable to luminous intensities of various wave-lengths it is relatively impermeable to radiant energy, thereby protecting the egg against heat injury. This is contrary to the traditional function ascribed to the jelly as a device to focus the rays of the sun and increase the temperature of the egg.

3. There is marked sensitivity to radiations at gastrulation expressed in high percentage of cases of spina bifida and failure of the blastopore to close. Many cases of spina bifida reported in the literature as due to Ultra-violet rays may be due to specific heat injury of the dorsal lip by the Infra-red or the entire spectrum of the mercury arc used. This suggestion is supported by the fact that these abnormalities can be induced by monochromatic radiations of any wave-length providing the radiant energy is sufficient.

4. The black pigment of the Frog's egg or embryo is not sensitive to various luminous intensities or wave-lengths if the total energy is constant. The absorption efficiency of this pigment toward radiant energy will shortly be measured.

5. From the embryological point of view the radiant energy factor is the most important. This probably applies also to forms which are not so efficiently pigmented.

6. Yung's results, showing the specifically deleterious effect of monochromatic green and the beneficial effects of violet, blue and red in respect to tadpole growth rate, can be attributed to the technical difficulties which were encountered in

1881 as well as to the fact that he did not realize that the plant food of the tadpoles had a survival and growth curve under colored lights similar to that of his tadpoles. Also, Yung's filters had a minimum transmission in the green in respect to all three radiation factors, namely: luminous intensity, quality, and radiant energy. This would partially explain the deleterious effect of green on the tadpoles as secondary to the effect on tadpole food. This statement would also apply to the beneficial effects of violet, blue and red.

7. The various radiation factors must be more rigidly calibrated and controlled in biological experiments so that no longer will luminous intensity and radiant energy be confused, or Ultraviolet mean the total radiation from a mercury arc.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 22).

VARIEGATED EYE COLOR IN THE PARASITIC WASP HABROBRACON

DR. ANNA R. WHITING

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The semi-dominant factor shot-veins (sv) arose simultaneously in three different lines after extreme heat treatment of larvae. It causes veins of the wings to be broken up and distorted. Shot-veined stock has proved to be fully fertile and stable. In connection with studies of linkage made in 1931, shot-veins was crossed to white eyes and the F_1 daughters (Whwh.Svsv) bred as virgin. Wild-type (Wh.Sv), white (wh.Sv), and shot-veined (Wh.sv) males appeared in expected ratios and with them the double mutant type (wh.sv) with shot-veins and white eyes, the latter all showing a mottling of red spots in the posterior ventral region. These spots always appear in both eyes. Their distribution and intensity show some variation but they can never be seen from the dorsal side. The homozygous white shot-veins stock is called variegated. It has been followed for almost two years and has been found to breed true and to be perfectly viable, in haploid males as well as in their diploid sisters. When white-eyed females are heterozygous for shot-veins variegation is present but less extensive.

Combinations have been made of white and shot-veins with the other eye color mutants but in no cases does the variegated condition occur except with three of the four allelomorphs in the orange locus, type (O), dahlia (o^d), and orange (o), and with the carrot allelomorph to white. Amount of spotting and intensity of color decrease in the orange series from type to orange.

Shot-veined females having the factor for white eyes and for its incompletely recessive allelomorph carrot ($whwh^c$.svsv) show the variegation on a cream background. Such females heterozygous for shot-veins ($whwh^c$.Svsv) show slight variegation on cream background.

Several investigators have reported mottling effects in eyes of Drosophila but none so far reported resembles the type of variegation here described which always shows in the same region, is perfectly stable and fully viable in homozygous and azygous (haploid) condition.

The dominance of variegation over non-variegation suggests that shot-veins is the result of a stable translocation. The possibility of its being a dominant gene mutation is not ruled out, however. The reason for the appearance of red spots in the double mutant type, white shot-veins, is not easy to give with any degree of assurance. It is suggested that the shot-veins factor or condition is associated with the constant habit of somatic mutation occurring at a definite stage in the development of the compound eye, such that only facets in the posterior ventral region are affected. This would make every eye a mosaic. Mosaicism involving always the same small area of somatic tissue and never the gonads would be required to explain it. It is likewise possible, and to the author probable, that the shot-veins condition or gene has a spotting effect on the eyes when the residual heredity is such as to allow its expression and that the cells of the red region are of the same genetic constitution as those of the white.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 29).

EGG-TRINUCLEARITY IN HABROBRACON

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The majority of mosaics in Drosophila have been shown to result from chromosome elimination in early development. A few examples have appeared however which must be explained by egg-binuclearity.

According to the various theories of egg-binuclearity, the two nuclei from which the embryo develops differ from each other in hereditary constitution. Thus the parts of the body descended from one cell differ from the parts tracing back to the other.

Morgan (1905) suggested that sex-mosaics (gynandromorphs) in bees might arise if the reduced egg nucleus were fertilized by one sperm nucleus, while a second sperm nucleus segmented independently. Boveri (1915) held that one of the first two blastomeres of a parthenogenetically dividing egg might be fertilized while the other continued in parthenogenetic cleavage. Doncaster (1915) obtained cytological evidence for fusion of two separate eggs of Abraxas. The nuclei of each underwent normal reduction, resulting in a binucleate egg with nuclei of different composition. Whiting (1924) assumed that in the origin of male mosaics of Habrobracon, the second polar nucleus as well as the mature egg nucleus took part in parthenogenetic cleavage. Goldschmidt (1931) presented both cytological and genetic proof of this hypothesis for Bombyx except that both nuclei were fertilized, there being no parthenogenesis.

Over 300 mosaics have thus far been obtained in Habrobracon and it has hitherto been assumed that these have been developed from binucleate eggs according to the hypothesis proposed in 1924. Evidence has now accumulated that in certain cases at least mosaics arise from trinucleate eggs. It may be supposed that three of the four nuclei from one unreduced egg nucleus (oöcyte) function in the development of the mosaic embryo. As an example of a mosaic arising from a trinucleate egg the following may be cited: A virgin female heterozygous for honey body color and for stumpy legs produced in addition to the expected wild-type, honey, stumpy, and honey stumpy males, a mosaic male with three legs wild-type, one leg stumpy and two legs honey stumpy. Raymond J. Greb has now obtained several cases of similar trinucleate mosaics. Kathryn A. Gilmore was the first to find an example which must be explained by egg-trinuclearity. In 1931 she obtained a mosaic male among the progeny of a female heterozygous (c.l/n.d) for the linked genes cantaloup, long, narrow and defective. The left eye was wild-type (black) while the right was cantaloup. Left wings were narrow, right were long defective. Breeding test showed that some of the sperm were cantaloup long defective while some were wild-type in all respects. Thus at least three combinations of the maternal genes were present in the mosaic; +, n, and c.l.d.

It is altogether possible that four different combinations of the maternal factors may occur in a male mosaic, but such a condition has not yet been found. If more than four combinations should occur or if one member of an allelomorphic pair should be found in association with three different combinations while the other member should likewise be present, it would prove that the mosaic developed from more than one unreduced egg nucleus, thus favoring the hypothesis of Doncaster.

It is highly probable that many of the mosaics previously explained by egg-binuclearity may have come from trinucleate or quadrinucleate eggs, but evidence for this would be obtainable only if the mother were heterozygous for more than one gene affecting the same structure. Thus far breeding tests of mosaic males have shown gonads to consist of not more than two of the possible combinations of factors, but embryological studies of the origin of the gonads indicate that more than two combinations are possible.

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The Collecting Net

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IMPRESSIONS OF THE THIRD INTERNATIONAL CONGRESS FOR EXPERIMENTAL CYTOLOGY AS OBTAINED FROM PROFESSOR CHAMBERS[1]

In spite of the fact that the General Secretary of the Third International Congress of Cytology, Dr. Rhoda Erdmann, who is editor of *Archiv Für Experimentelle Zellforschung,* was incarcerated for three weeks in one of Hitler's concentration camps near Berlin, the meetings of the Congress were a great success. One of the unexpected results of the Congress was her release so that she need not function by proxy.

The Congress was one of the most successful of its kind largely due to the homogenity of interest of a not too large a group of persons interested in the functional activities of the cell. There were about 130 attending the meetings, and the excellent planning out of the social activities during the week made all of its members come to know each other well by the end of the week.

The evening functions were (1) a reception at Kings College by Dr. Gray, local President of the Congress, and Mrs. Gray. (2) A motion picture presentation of scientific films in the auditorium of the newly erected physiology laboratory of Professor Barcroft. (3) A dinner in the Hall, replete with traditions, of Trinity College. (4) Last, but by no means least, a delightful dancing party at the famous Dorothy Café, at which the ladies of Cambridge served as hostesses.

The Congress was opened by the Mayor of Cambridge, Mrs. Keynes, mother of J. Maynard Keynes, the distinguished English economist, and mother-in-law of Professor A. V. Hill. Mayor Keynes also graced the Congress with her presence at the dinner in Trinity and at the Presentation of the films.

Among the Americans present were: Drs. Harrison, Streeter, Brooks, Lund, Hope Hibbard, Mary J. Hogue, Chambers, Speidel, Beutner, Raymond Parker, and H. Pinkerton. All European nationalities were well represented, including Soviet Russia.

[1] Dr. Chambers and Dr. Carrell served on the organizing committee appointed by the previous Congress which convened in Amsterdam in 1930. The former presented a paper at Cambridge on "Some features of cell permeability in relation to kidney function."

Selected topics for the meetings gave a well rounded picture of cellular physiology.

The first day was devoted to cell respiration and metabolism which included metabolism in plant tissues, bacteria and amphian ova.

The second day dealt with the relation of cellular structure to function.

Electro-physiology, the topic of the third day, was started by Professor Adrian who made a unique demonstration which enabled the audience to hear, in the form of varying booming sounds, the response of successive muscle fibres through a needle electrode inserted into the fleshy part of his own arm.

The fourth day dealt principally with developmental mechanics in which one of the interesting papers was that of Dr. and Mrs. Needham and C. H. Waddington who presented evidence of having obtained cell-free aqueous extracts, possessing organizer-activity in early embryonic development. The Golgi apparatus furnished a topic for considerable discussion in which Irish eloquence played a prominent rôle.

The fifth day dealt with animal and plant viruses, a topic which stimulated interest among the cytologists because of observable effects in the nuclear and cytoplasm of living cells caused by the activity of viruses.

One of the many interesting motion pictures was that of Dr. F. M. L. Sheffield of Herpendon in which the progressive increase of coagulum-like lumps could be observed forming in the protoplasmic strands within hair cells of a plant which had been infected with a virus disease.

Each afternoon tea was served in the Department of Pathology. A large series of very interesting demonstrations were open to view in an adjoining room. Of especial interest were slides of Dr. Holtfreter from Spemann's laboratory which showed the organizing activity of pieces of a variety of foreign tissues planted under the skin of larval frogs.

A very significant demonstration of the physical properties of protein films was that of E. K. Rideal, professor of physical chemistry at Combridge, in which he showed the effects of enzyme action in changing their properties.

A new and simple form of a micro-manipulator to be used with the ultrapak microscope was demonstrated by Dr. Himmelweit, who has been given an asylum in St. Mary's Hospital because of his sudden and forcible expulsion from the Pathological Institute in Berlin.

There were several conducted parties to points of historical interest such as the colleges, Ely Cathedral, etc., the attraction of which proved to be a sore temptation for some members to absent themselves from meetings.

Among those who took active part in the arrangements of the Congress were Dr. and Mrs. Shearer. Dr. Shearer, who is well known for his contributions to experimental embryology, knows the Marine Biological Laboratory from the days of its inception for he was a student of the late Professor Whitman and took part in many of the pranks instigated by the youngsters of those days such as E. B. Wilson, Jacques Loeb, T. H. Morgan, etc. He was present when Professor Whitman rescued the Laboratory from its financial difficulties at the time when influential Bostonians sought to destroy it.

For the extraordinary success of the running of the Congress credit is due to the members of the Strangeway's Research Laboratory, especially to Dr. Honor B. Fell and Dr. F. G. Spear. Dr. Fell visited Woods Hole last summer. Mention must also be made of the services of Professor D. Keilin, professor of cellular biology at Cambridge, Dr. and Mrs. Needham of Sir Gowland Hopkins' Laboratory, and Dr. R. A. Webb of the Department of Pathology at Cambridge.

Dr. and Mrs. Gray, well known to those of us at Woods Hole, together with their enthusiastic group of colleagues, are to be congratulated in making the Third International Congress for Experimental Cytology one of the most successful of its kind. Their delightful hospitality will long be remembered. The perfect weather, the beautiful gardens, and the uniqueness of the old university town of Cambridge, combined with the other features, made the visit there an unforgettable occasion.

A full report of the papers presented together with discussion of the papers will be printed in Erdmann's journal.

Dr. Chambers had as his companion on the train from London to Cambridge a prominent German biologist who was thrown out of his position as Privat-dozent at a German University. During their conversation on the train Dr. Chambers learned that he was in the tropics during the Great War, but that he rushed back to his Fatherland to join the conflict. He went through the entire period of the war in which he distinguished himself and was awarded the Iron Cross. He carried with him his honorable discharge from the Army. He was a member of one of the time-honored student corps during his university career and had prominent scars on his forehead and cheeks as testimony of numerous sabre contests. He is a typical Prussian in appearance, but because one of his grandmothers had Jewish blood he was thrown out of his position in one of the German universities. His only recompense for his war service is permission to start up medical practice where and when he can.

ITEMS OF INTEREST

Among the investigators still in Woods Hole on Monday, September 18 were: W. R. Amberson, Louise S. Armstrong, P. B. Armstrong, G. A. Baitsell, L. G. Barth, L. V. Beck, Louise E. Boydon, G. N. Calkins, W. Cattell, R. Chambers, T. T. Chen, F. E. Chidester, Eleanor Clark, E. R. Clark, Frances Clark, G. H. A. Clowes, E. G. Conklin, S. A. Corson, Margaret Crane-Lillie, K. Dan, W. L. Doyle, Dorothy Francis, H. J. Fry, W. E. Garrey, Ethel B. Harvey, E. N. Harvey, L. V. Heilbrunn, Ella N. Hoppe, H. E. Howe M. H. Jacobs, J. M. Johlin, Takeo Kamade, J. B. Katz, Anna K. Kelch, F. R. Lillie, R. S. Lillie, Ruth S. Lynch, S. O. Mast, A. P. Mathews, J. A. Miller, F. B. Moreland, Lilian V. Morgan, T. H. Morgan, Helen K. Newton, J. F. Nonidez, C. Packard, G. F. Papenfuss, S. E. Pond, G. S. de Renyi, Florence M. Scott, F. M. J. Sichel, H. Specht, H. B. Steinbach, C. R. Stockard, O. S. Strong, Miss E. M. Vicari, Lucille W. Wade, and Edith M. Wallace.

Dr. R. F. Pitts, who took the course in physiology here in 1930, has recently been appointed instructor at the New York University Medical School.

Dr. Kenneth S. Rice was recently appointed as the head of the department of zoology at the University of Maine, where he has worked for the last six years, succeeding Dr. D. B. Young who has accepted a position at Washington University.

Dr. Ancel B. Keyes has been appointed instructor in the biochemical sciences in the Fatigue Laboratory of Harvard University. Dr. Keyes has held a National Research Council Fellowship for the past two years, working in 1930-31 in Copenhagen at the Laboratory of Zoophysics under Dr. Krogh, and in the following year under Dr. Barcroft at Cambridge.

The Imperial University of Tokyo has delegated Mr. Takeo Kamade to visit the marine laboratories of the United States in order to inspect their equipment with a view to aiding the planning of similar laboratories in Japan. Mr. Kamade is now visiting Woods Hole.

Dr. Ralph A. Kekwick, who has been studying for the past two years at New York University and Princeton, occupied the last few months of his tenure of a Commonwealth Fund Fellowship at Woods Hole in the investigation of the permeability of *Arbacia* eggs under anaerobic conditions. He has returned with Mrs. Kekwick to the University of London, where he will be engaged in teaching biochemistry.

THE EFFECT OF X-RAYS UPON CELL OXIDATIONS

Dr. Leon C. Chesley
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Exposure of young organisms to sufficiently large doses of X-rays inhibits subsequent development. In considering the primary seat of attack of radiation in the cell, it is only natural that attention should be directed to the energy releasing mechanisms; of these, respiration is predominant.

The view is commonly held that the flux of activity—so characteristic of living protoplasm—requires free energy. In the cell, free energy is obtained chiefly by the processes of oxidation. Hence to study fundamental life processes, study respiration and factors controling it, as respiration is one of the ultimate processes governing vital activity.

Loeb proposed that the essential feature of fertilization is the increasing rate of oxygen consumption which causes or permits development to proceed. This hypothesis is based upon certain of his findings in the sea urchin egg. The literature is reviewed and discussed at length by Whitaker (J. Gen. Physiol. 16:497).

It seemed profitable to investigate the effect of radiation upon respiration, particularly as iron is so important in the respiratory process. Presumably iron exerts its catalytic effect by alternate oxidation and reduction, i.e. by the gain and loss of electrons. The primary action of X-rays is to ionize the atoms and molecules in the permeated medium by this very process of electronic change; the action is most marked on the heavy elements, such as iron.

It was thought that some light might be shed upon the problem of development by a study of X-ray effects upon respiration and development concomitantly. There is but little in the literature which bears directly upon this question. Most of the work dealing with radiation effects upon respiration has been done on mature differentiated cells. Recorded results are discordant.

Hubert (Pflüger's Arch. 223:333) found that exposure of chick embryos to X-rays affected growth before glycolysis.

Adler (Strahlentherapie 36:1) and Crabtree (Report Imp. Cancer Res. Fund 10:33) demonstrated that while respiration is finally influenced by irradiation, there is a latent period of eight to twelve hours before any effect is noticed.

Redfield and Bright (J. Gen. Physiol. 3:297) studied the effects of radium emanation upon CO_2 production and growth in the radish seed. They found that "changes in the rates of CO_2 production and cell division do not always go hand in hand. One is increased by exposures which retard the other."

In the present study, the oxygen consumption was determined at different times after irradiation in wheat seedlings and the eggs of *Arbacia* and *Chaetopterus*. The anaerobic metabolism of the eggs was studied as well. All measurements were made manometrically, using the Barcroft-Warburg apparatus.

In the wheat seedling experiments, the dose of X-rays used (2260 r) was sufficient to inhibit growth by forty per cent., as measured twenty-four hours after irradiation. The routine employed in preparing the seedlings for irradiation has been described by Failla and Henshaw (Radiology 17:1). Wheat seeds were soaked for an hour and placed into moist chambers at 26°C for eighteen hours in the dark. The seedlings were then selected for uniformity, irradiated, and put into moist chambers which were kept at 26°C or 6° C. Respiration was determined 4, 24, 48, and 72 hours after irradiation. Fresh weight was determined by stripping the sprout from the grain and weighing at once.

Arbacia eggs were obtained by cutting the animal in two equatorially and inverting the upper half in a syracuse watch glass; mature gametes are then shed. The eggs were washed and concentrated to one volume in forty of sea water, and irradiated (22,000 r).

Chaetopterus eggs were collected in the usual way and treated in the same manner.

Anaerobic metabolism was studied by adding methylene blue (final concentration 0.005 per cent) to the egg suspensions. Barron (J. Biol. Chem. 81:445) states that the resulting increase in O_2 consumption is proportional to the anaerobic metabolism.

Under all conditions investigated, the results were the same. Irradiation sufficient to impede or stop growth has no effect upon cell oxidations.

While growth in the wheat seedling was inhibited forty per cent., the oxygen consumption per gram fresh weight was the same in control and irradiated samples. This result was corroborated in another way. The seedlings kept at 6°C did not grow perceptibly. When the oxygen consumption for this series was determined, it was found to be the same in control and irradiated seedlings whether calculated on the basis of fresh weight or per seedling.

In the cases of the marine eggs, the doses given were sufficient to kill the embryos after a

period of a day or two. Early development was seriously hampered. In the *Arbacia* eggs, cleavage was considerably delayed and abnormal cleavages were frequent. The largest dose used (65,000 r) was almost immediately lethal to *Abracia* eggs.

No effect upon respiration was found.

All measurements of respiration of eggs were made within six hours of irradiation. This should detect any direct influence that irradiation might have on respiration. Later changes in metabolism are almost certainly secondary, if, indeed, they occur at all.

As for anaerobic metabolism, no constant results have been obtained. However, there is apparently no influence of radiation upon this phase of cell activity.

These experiments do not prove that growth and development are independent of respiration. Development may depend upon several factors, concomitantly or concatenately, of which respiration is but one. One or more of these factors is radiosensitive to such a degree that doses of X-rays may stop development without affecting respiration.

We can conclude only that respiration certainly, and all of the energy releasing mechanisms probably, are not the seat of radiation attack in the cell. If, and when, respiration is affected by irradiation, it is a secondary reaction.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 22).

BOOK REVIEW

Science and Sanity. An Introduction to Non-Aristotelian Systems and General Semantics. Alfred Korzybski. *Ca.* 800 pages. $7.00. The Science Press, 1933.

This interesting and original book deserves careful study by all who are concerned with science and its applications. It is unusually comprehensive in its scope, and any adequate survey of its contents would require a much fuller review than is here possible. Broadly speaking, two main fields of general scientific interest are covered: first, the methods, aims and presuppositions of scientific method in general; and, second, the applications, actual and possible, of science in the individual and social life of human beings. Although not primarily biological, the book is pervaded by the biological conception of life as consisting primarily in the adjustment of organism to surroundings. The failures and difficulties of modern society are largely the sign of deep-seated biological maladjustments which it would be quite possible to correct by the intelligent and systematic application of existing scientific knowledge.

The methodological part of the book is remarkable chiefly for the author's vigorous advocacy of what he calls "non-Aristotelian" methods of logical procedure. He exposes clearly the fallacies which arise from a too exclusive dependence on reasoning of the classificatory or syllogistic type derived from Aristotle, with its tacit assumption of the equivalence or essential identity of all units belonging to a given class. Individuals of the same class usually receive the same verbal designation or name; and being thus identical in name it is automatically taken for granted that they are identical in all other respects. False identifications and failures of discrimination inevitably follow. The author wages a vigorous polemic against the habits of non-discrimination based on the unreflecting acceptance of purely verbal reasoning. He shows that whenever there is an unconscious or automatic identification of the symbol (word, image, diagram, formula) with the thing symbolized, fallacies of this type are likely to arise. Verbalism, with its many pitfalls and confusions, is clearly analyzed. Evidently the affixing of the same label to two different objects does not render them identical in their actual properties and activities. The only permanently valid, i. e., scientific, procedure is to face the reality in an objective and disinterested spirit and not be deflected from a realization of its true character by the representative signs employed. "Words are simpler and take less effort to handle than objects." (p. 480) But real things "are what they are"; their existence is prior to their designation; real existence is always indefinitely complex; hence all mental representation of real objects, including scientific representation, necessarily involves abstraction, *i. e.*, a singling out of partial features or aspects for special or exclusive attention and a neglect of other aspects. On the objective level things have their own actuality which is only partially representable. In other words, in all mental representation of reality—*i. e.*, in all knowledge—certain characters of the reality are "left out" of consideration. The truly educated person is conscious of this; the verbalist is not; he attaches more importance to the sign or word than to the actuality. Thus *being* a liar or thief seems to the unregenerate man a matter of less importance than being *called* one; he is hugely indignant at having the label attached, while having little or no objection to exemplifying the deplorable actuality in his own

person. This is one illustration of the manner in which verbalism deflects attention from reality and so makes for confusion and falsification, with often disastrous consequences. As Count Korzybski puts it (p. 486), "the ignorant or pathological use of language is a public danger." As a countermeasure he would have children early trained in habits of discrimination, based on close observation of actual things rather than on the study of language alone. They should be made to realize that things exist on their own objective or "unspeakable" level and have their own obstinate reality to which we must learn to adjust ourselves. In his dealings with things, the educated person should be "conscious of abstraction"; naming, classifying and reasoning necessarily involve abstraction and hence incompleteness, and he is always aware of this. Such a person is immune to the virus of verbalism. The basis of true knowledge is discrimination—not the equalization or identification of differents. The importance of this "principle of non-identity" is insisted on throughout the book.

The legitimate use of abstraction, for the purpose of gaining real insight into and control over actuality, is the special province of science; and the nature of scientific abstraction is considered at length, with a wide range of illustrations from mathematics and the natural sciences. The discussion of mathematical procedure and its application in the sciences of nature is very full and clear. Mathematics gives the most exact and complete account of the purely formal or structural side of nature, *i. e.*, of the orderly and permanent conditions exemplified by all phenomena. Permanently valid or scientific knowledge is always knowledge of structure and relations rather than of complete reality. Such knowledge consists essentially in a formal correspondence, best expressed mathematically whenever possible, between the representation and the reality represented. Since knowledge is correspondence or adjustment, we should expect to find certain definite parallels between the structure of the nervous system (the chief physiological instrument of adjustment) and the general structure of the external world; and in an interesting section of the book these parallels are described and discussed. The fundamental problems of physical science have reference to the general or foundational characteristics of world-structure; and in the concluding section the author gives a concise and lucid account of the elements of quantum theory and relativity.

The applications of science to the general problems of human society meet with the obstacle that many classifications and social procedures, which to the objective and disinterested view of science appear fundamentally fallacious, are based on conceptions of long standing which are sanctioned or stabilized by language and traditional usage. Hence they become the objects of misplaced and often passionate loyalty. There is also the general human tendency to undue simplification, and to action based on over-simple conceptions reinforced by verbalisms. Men are actuated by language and tend to identify and to treat alike all things that are similarly labeled. Other important fallacies are based on identity of money value; men regard as equally valuable and desirable all things which have the same price, or all activities which have the same remuneration. For permanent social progress a far-reaching revision of concepts is required; this revision requires in its turn an extensive program of reform in education, economics and government. It is largely for lack of sound scientific control that political, social and economic affairs meet with such frequent collapse or failure. As the author remarks toward the end of his book (p. 538): "world affairs have seemingly come to an impasse, and probably without the help of scientists, mathematicians and psychiatrists included, we shall not be able to solve our urgent problems soon enough to prevent a complete collapse."

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SCIENCE AND PROGRESS

(*Continued from Page* 356)

nesses, the duel, and a thousand other customs of savagery and barbarism, is due not to eugenics, but to education—and war can be banished by the same means.

Fortunately the opportunities for world wide education were never so good as they are today. The printing press, the telegraph, the telephone, radio, moving pictures, rapid transit on land and sea or in the air, have put information concerning the whole world within the reach of everyone. World opinion can now be formed and expressed, not years and centuries after an event, but while it is happening. No nation can long stand against the sober judgment of the majority of mankind. Japan and Germany are showing that they are sensitive to world opinion. There is great force in what our Declaration of Independence has so well expressed in the phrase, "A decent respect to the opinions of mankind."

This is no easy or rapid cure for the ills of the world, but it is the only rational one. Science and education, knowledge and ethical character, are the chief hopes of human progress.